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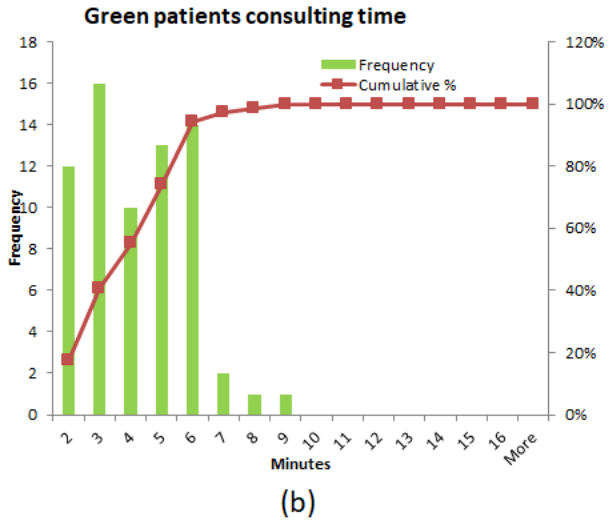
SECTIONS

ARTICLES



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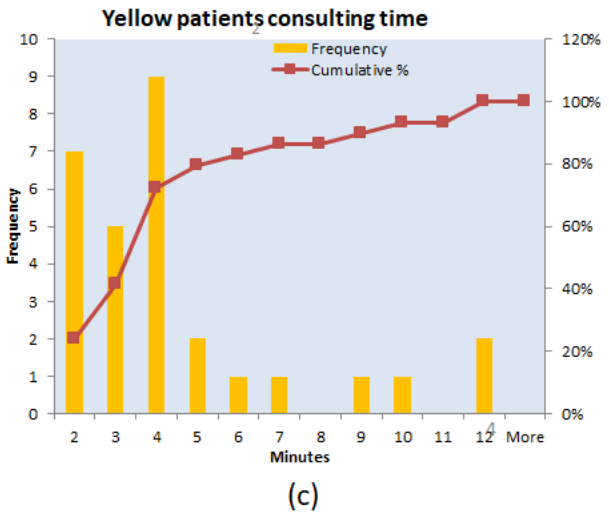

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DISTRIBUTION OF THE NUMBER OF ARRIVALS DURING THE DAY AND TIME SPENT DURING A CONSULTATION FOR YELLOW AND GREEN LEVELS IN THE EMERGENCY DEPARTMENT OF RECANTO DAS EMAS, FEDERAL DISTRICT, BRASIL.

(b) time spent during a consultation for green level, and (c) time spent during a consultation for yellow level.

RAMB REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

SUMMARY

SECTIONS

ARTICLES

REVIEW ARTICLES

Hypertension and cholesterol

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Cardiovascular aggravators result from a series of factors that may be related among themselves or metabolically connected; thus, these may contribute to and determine the onset of future diseases that may affect the functioning of the heart, such as arterial hypertension (SAH)[1]. SAH is frequent in developing and developed countries, and the number of occurrences increases with age[2]. There are, in Brasil, 250,000 deaths per year due to cardiovascular diseases, and SAH is one of the causes in approximately 50% of them[3]. Studies show that its occurrence is between 52% and 63%, which makes it possible for SAH to be considered a public health problem that considerably increases the cardiovascular risk of patients in these cases[1,4-6].

Clinical trials for the management of hypertension show the importance of controlling blood pressure (BP) as a means of reducing the risks of cardiovascular diseases[3]. A review study that included eight trials and more than 15,000 individuals aged 60 years or older indicated that the use of antihypertensive agents reduced stroke by 30%, coronary heart disease by 23%, and mortality by 13%. Patients with SAH have obesity, elevated heart rate, diabetes mellitus, and high cholesterol levels. SAH alone is found in only 13% of men and 20% of women[7]. This shows the importance of detecting, controlling, and treating other aggravating factors, if present, early.

SAH is characterized by several functional and structural changes in the plasma membrane, which are constantly related to changes in metabolism, such as high blood triglycerides, low levels of HDL, and high levels of LDL[8]. The elevation of viscosity in the plasmalemma that occurs in high-pressure carriers shows changes in lipid composition[9]. In cases of high levels of triglycerides and high levels of cholesterol, there is a great transition between the lipids present in the blood and those present in the plasma membranes, which leads to a decrease in the fluidity of the membranes and a change in the transport of ions[8]. An increased supply of cholesterol to plasma membranes was associated with decreased sodium and potassium pump function, reduced sodium efflux, and increased intracellular sodium affinity[10,11]. The decrease in plasma membrane cholesterol has increased the rate at which sodium and potassium pump ions are transported[12,13]. Similarly, the decrease of plasma membrane cholesterol in erythrocytes led to increased sodium efflux and decreased intracellular sodium[14]. The latter may be considered beneficial since the reduction in the sodium levels present inside the cells is an alternative for the prevention and treatment of cardiovascular diseases[15].

Studies show that in people without pre-existing cardiovascular diseases, the use of statins in patients with high lipid levels leads to a reduction in cholesterol

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levels with a consequently reduced occurrence of cardiovascular diseases[16]. In Brasil, there has been an increase in the consumption of processed and industrialized foods in recent years, with a reduction in the consumption of healthy foods such as vegetables and legumes[17,18]. This contributes to the elevation of the levels of saturated fat, trans fat, and sugar consumed, leading to an increase in cholesterol levels[19,20], which is associated with diets with high sodium content, which in turn accentuates the increase in BP, thus contributing to the increase of chances of developing hypertension. Thus, it is important to raise the population's awareness of the need to have a healthy diet, since food can determine the emergence of new diseases and comorbidities that, associated or not with other pre-existing factors, can lead to a decrease in health and quality of life.

Work conducted in the city of São Paulo, both authors (Rubens Moura Campos Zeron and Victor Campos de Albuquerque) participated in the reference search, drafting, and revision of the paper.

REFERENCES

1. Marte AP, Santos RD. Bases fisiopatológicas da dislipidemia e hipertensão arterial. Rev Bras Hipertens. 2007;14(4):252-7.
2. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003;289(19):2560-72.
3. Miranda RD, Perrotti TC, Bellinazzi VR, Nóbrega TM, Cendoroglo MS, Toniolo Neto J. Hipertensão arterial no idoso: peculiaridades na fisiopatologia, no diagnóstico e no tratamento. Rev Bras Hipertens. 2002;9(3):293-300.
4. Oliveira SMJV, Santos JLF, Lebrão ML, Duarte YAO, Pierin AMG. Hipertensão arterial referida em mulheres idosas: prevalência e fatores associados. Rev Text Context Enferm. 2008;17(2):241-9.
5. Jobim EFC, Cabrera MAS. Prevalência de hipertensão arterial em idosos atendidos no programa da saúde da família em Jardim Alegre, PR. Rev Envelhec Saúde. 2007;13(2).
6. Jobim EFC. Hipertensão arterial no idoso: classificação e peculiaridades. Rev Bras Clin Med. 2008;6:250-3.
7. Rudnichi A, Safar M, Asmar R, Guize L, Benetos A. Prevalence of cardiovascular risk factors in a French population. J Hypertens Suppl. 1998;16(1):S85-90.
8. Dominiczak AF, Davidson AO, Bohr DF. Plasma membrane in hypertension: microviscosity and calcium stabilization. Hypertens Res. 1994;17(2):79-86.
9. Yeagle PL. Lipid regulation of cell membrane structure and function. FASEB J. 1989;3(7):1833-42.
10. Lijnen P, Petrov V. Cholesterol modulation of transmembrane cation transport systems in human erythrocytes. Biochem Mol Med. 1995;56(1):52-62.
11. Levy R, Hevroni D, Cabantchik ZI, Livne A. Lii-Nao countertransport and Li leak in erythrocytes are differential1y affected by membrane enrichment with cholestetyl hemisuccinate. Biochim Biophys Acta. 1986;854(2):325-8.
12. Giraud F, Claret M, Garay R. Interactions of cholesterol with the Na pump in red blood cells. Nature. 1976;264(5587):646-8.
13. Claret M, Garay R, Giraud F. The effect of membrane cholesterol on the sodium pump in red blood cells. J Physiol. 1978;274:247-63.
14. Lijnen P, Celis H, Fagard R, Staessen J, Amery A. Influence of cholesterol lowering on plasma membrane lipids and cationic transport systems. J Hypertens. 1994;12(1):59-64.
15. Medina AJ, Pinilla OA, Portiansky EL, Caldiz CI, Ennis IL. Silencing of the Na^+/H^+ exchanger 1(NHE-1) prevents cardiac structural and functional remodeling induced by angiotensin II. Exp Mol Pathol. 2019;107:1-9.
16. Yusuf S, Bosch J, Dagenais G, Zhu J, Xavier D, Liu L; HOPE-3 Investigators. Cholesterol lowering in intermediate-risk persons without cardiovascular disease. N Engl J Med. 2016;374(21):2021-31.
17. World Health Organization. Integrated prevention of noncommunicable diseases: global strategy on diet, physical activity and health. Geneva: World Health Organization; 2004.
18. Levy-Costa RB, Sichieri R, Pontes NS, Monteiro CA. Disponibilidade domiciliar de alimentos no Brasil: distribuição e evolução (1974-2003). Rev Saude Publica [Internet]. 2005;39(4):530-40.
19. Willett WC. Surprising news about fat. In: Willett WC, ed. Eat, drink and be healthy: the Harvard Medical School guide to healthy eating. New York: Simon & Schuster Adult Publishing Group; 2001. p.56-84.
20. Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. N Engl J Med. 1999;340(25):1994-8.

Advanced non-small cell lung cancer – Treatment with Pembrolizumab

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize producers to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

Lung cancer is the worldwide leading cause of cancer-related deaths in both genders. Pembrolizumab is a IgG4 monoclonal antibody, which is highly selective, humanized, and binds to the programmed cell death protein 1 (PD-1) receptor and blocks its interaction with the PD-L1and PD-L2 ligands. We conducted a systematic review of the literature, without time restrictions, in the Medline database, using the following PICO: Adult patients with advanced NSCLC; Treatment with pembrolizumab alone or associated with chemotherapy, compared with chemotherapy, except for monochemotherapy with docetaxel and death outcomes (due to any cause) and adverse events. We selected 244 papers to answer the clinical question. The details of the methodology and the results of this guideline are set out in Annex 1.

INTRODUCTION

Lung cancer is the worldwide leading cause of cancer-related deaths in both genders, and smoking is its main etiological factor. The discovery of immunological checkpoints corroborates the hypothesis that ligands present in the tumor modulate the carcinogenesis mechanisms and immune activity of the tumor microenvironment. Among the most studied coregulatory molecules, the PD-1 (programmed cell death 1) and its ligand PD-L1 (programmed cell death ligand 1) stand out.

The PD-1 is a immunologic checkpoint that limits the activity of T cells (lymphocytes) in peripheral tissues. The PD-1 pathway is a immunological control checkpoint that can be coupled by tumor cells to inhibit immune surveillance of the active T cell.

Pembrolizumab is a IgG4 monoclonal antibody that is highly selective, humanized, and binds to the programmed cell death protein 1 (PD-1) receptor and blocks its interaction with the PD-L1 and PD-L2 ligands, which are expressed in antigen-presenting cells and may be expressed by tumors or other cells in the tumor microenvironment, assisting them in preventing of their detection and elimination by the

immune system of the host.

Non-small cell lung neoplasms, without mutations in the epidermal growth factor receptor (*EGFR*) gene or rearrangement of the anaplastic lymphoma kinase (*ALK*) gene, with gene expression for PD-L1, are the target of pembrolizumab.

RESULTS

Our study population included a total of 2,877 patients with NSCLC, submitted to pembrolizumab therapy (N=791) and compared to chemotherapy (N=788), or pembrolizumab plus chemotherapy (N=748) compared with chemotherapy (N=550), and followed-up to measure the death and adverse events outcomes for 12 months (Table 1 - Annex I).

Regarding the bias risk of the five studies included, three of them were not double-blind[1-3], so the overall risk of the studies can be considered low (Table 2 - Annex I).

META-ANALYSIS

Two RCTs[1,2] present sufficient data to allow for a meta-analysis, considering results (number of deaths) up to 12 months, comparing pembrolizumab with chemotherapy in advanced NSCLC (Table 3).

TABLE 3. MORTALITY RATE IN 12 MONTHS - PEMBROLIZUMAB *VS.* CHEMOTHERAPY.

Study	Pembro Patients/ Events	Carboplatin + pemetrexed or paclitaxel Patients/Events	* Platinum-based chemotherapy Patients/Events
Mok TSK, et al. 2019 Keynote-042	637/272	637/321	
Reck M, et al. 2016 Keynote-024	154/115		151/117

The incidence of death was 48.92% (387 in 791 patients) in the Pembrolizumab group and 55.58% in the Chemotherapy group (438 in 788 patients). Pembrolizumab reduced the risk of death by 7% at 12 months [ARR 7%; 95% CI -12% to -2%; p=0.006; I^2=0%, NNT=15 (95% CI 9 to 56)], Figure 2.

For this comparison, we also evaluated the adverse events due to any cause, degree ≥3, in the population treated (Table 4).

TABLE 4. ADVERSE EVENTS (GRADE ≥3) - PEMBROLIZUMAB *VS.* CHEMOTHERAPY.

Study	Pembro Patients/ Events	Carboplatin + pemetrexed or paclitaxel Patients/Events	* Platinum-based chemotherapy Patients/Events
Mok TSK, et al. 2019 Keynote-042	636/113	615/252	
Reck M, et al. 2016 Keynote-024	154/41		150/80

The incidence of adverse events ≥3 was 19.5% (154 in 790 patients) in the Pembrolizumab group and 43.4% in the Chemotherapy group (332 in 765 patients). Pembrolizumab reduced the risk of adverse events by 24% at 12 months [ARR = 24%; 95% CI -28% to -19%; p< 0.00001; I^2=0%, NNT=4 (95% CI 9 to 5)], Figure 2.

Three primary assays[3-5] comparing pembrolizumab plus chemotherapy *versus* chemotherapy allow to combine the results and assess the risk of death in 12 months, as the outcome (Table 5).

In all three studies, patients were randomized (1: 1) in blocks of four, stratified by tumor proportion score of PD-L1 (<1% *vs.* ≥1%).

In the meta-analysis of the results of these three studies, there is a significant difference in the

FIGURE 2. COMPARISON FOREST PLOT: PEMBROLIZUMAB *VERSUS* CHEMOTHERAPY, OUTCOME: MORTALITY AT 12 MONTHS.

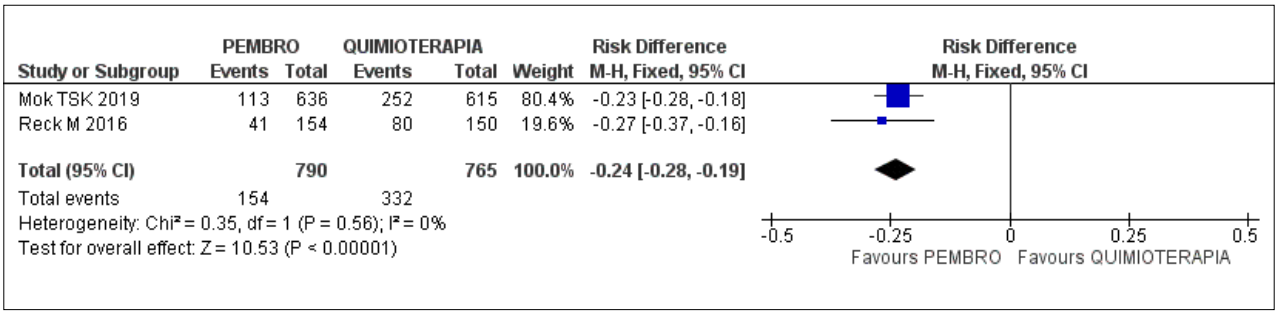


FIGURE 3. COMPARISON FOREST PLOT: PEMBROLIZUMAB *VERSUS* CHEMOTHERAPY, OUTCOME: ADVERSE EVENTS WITH A DEGREE GREATER THAN OR EQUAL TO 3.

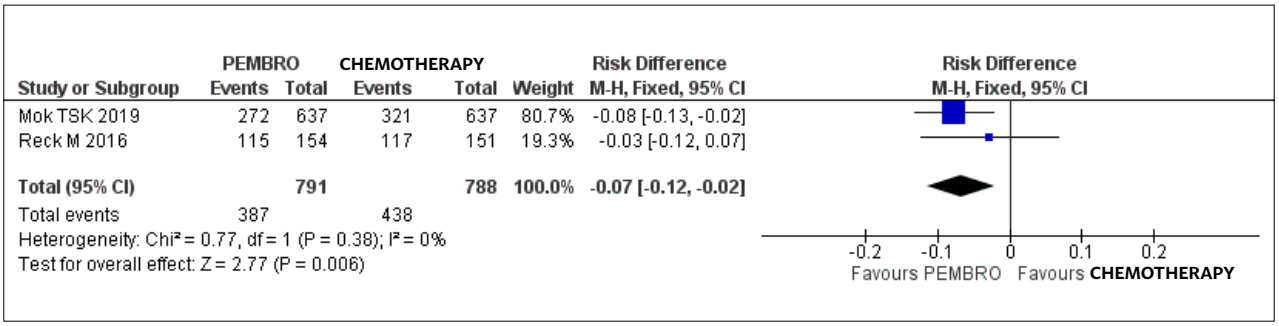


TABLE 5. MORTALITY - PEMBROLIZUMAB + CHEMOTHERAPY *VERSUS* CHEMOTHERAPY.

Study	Pembro + carboplatin + paclitaxel or nab-paclitaxel patients/ events	Pembro + cisplatin or carboplatin + pemetrexed patients/ events	Pembro + carboplatin + pemetrexed patients/ events	Carboplatin + paclitaxel or nab-paclitaxel patients/ events	Cisplatin or carboplatin + pemetrexed patients/ events	Carboplatin + pemetrexede patients/ events
Paz-Ares L, 2018 Keynote-407	278/216			281/236		
Gandhi L, 2018 Keynote-189		410/247			206/147	
Langer CJ, 2016 Keynote-021			60/27			63 / 32

FIGURE 4. COMPARISON FOREST PLOT: PEMBROLIZUMAB + CHEMOTHERAPY *VERSUS* CHEMOTHERAPY, OUTCOME: MORTALITY AT 12 MONTHS.

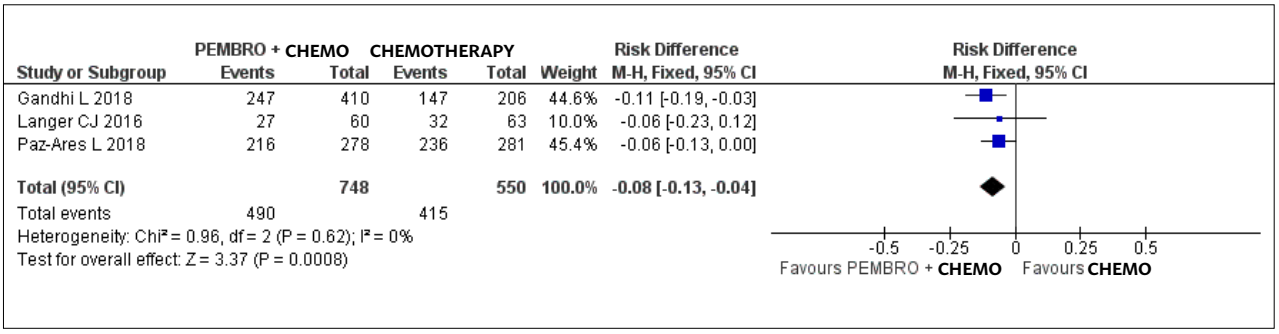


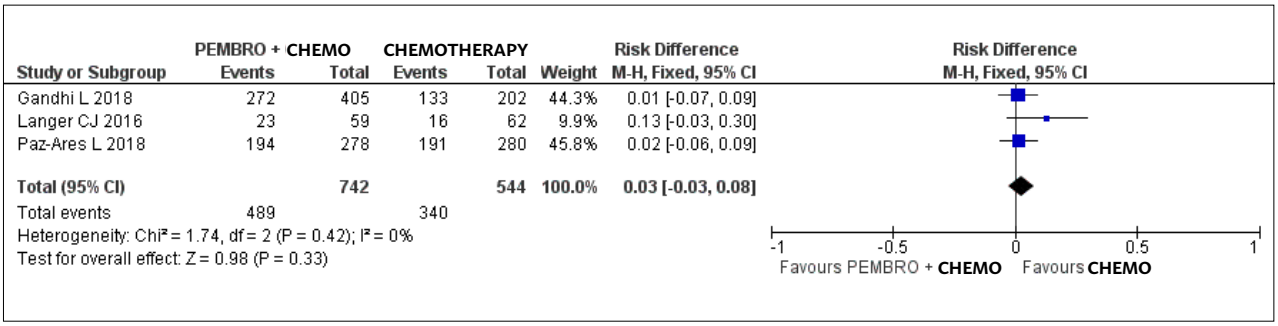
TABLE 6. ADVERSE EVENTS (GRADE ≥3) - PEMBROLIZUMAB + *CHEMOTHERAPY*

Study	Pembro + carboplatin + paclitaxel or nab-paclitaxel patients/ events	Pembro + cisplatin or carboplatin + pemetrexed patients/ events	Pembro + carboplatin + pemetrexed patients/events	Carboplatin + paclitaxel or nab-paclitaxel patients/ events	Cisplatin or carboplatin + pemetrexed patients/ events	Carboplatin + pemetrexede patients/ events
Paz-Ares L, et al. 2018 Keynote-407	278/194			280/191		
Gandhi L, et al. 2018 Keynote-189		405/272			202/133	
Langer CJ, et al. 2016 Keynote-021			59/23			62/16

reduction of death, in a 12-month follow-up, of 8% (95% CI -13% to -4%); favorable to the treatment with pembrolizumab plus chemotherapy; [I^2=0%; NNT=12 (95% CI 7 to 25)] - (Figure 4).

These three RCTs analyzed adverse events of any cause, degree ≥3 in the population treated, for comparison between pembrolizumab plus chemotherapy *versus* chemotherapy (Table 6).

FIGURE 5. FOREST PLOT COMPARISON: PEMBROLIZUMAB + *CHEMOTHERAPY VERSUS* CHEMOTHERAPY. RESULTS: ADVERSE EVENTS WITH A DEGREE GREATER THAN OR EQUAL TO 3.



VS CHEMOTHERAPY

In the meta-analysis of three combined studies, there was no significative difference in adverse events with a degree ≥3, in a 12-month follow-up, between both groups (ARR =3%, 95% CI −0.03 to 0.08; NNT=NS; I^2=0%) (Figure 5).

SUMMARY OF THE META-ANALYSIS RESULTS

PEMBROLIZUMAB VERSUS CHEMOTHERAPY

Result or Subgroup	Studies	Participants	Statistical Method	Estimated effect
Mortality in 12 months	2[4,5]	1,579	Difference in risk (M-H, Fixed, 95% CI)	-0.07 [-0.12, -0.02] NNT 15, 95% CI 9 to 56
Adverse events with degree greater than or equal to 3	2[4,5]	1,555	Difference in risk (M-H, Fixed, 95% CI)	-0.24 [-0.28, -0.1]

PEMBROLIZUMAB + CHEMOTHERAPY VERSUS CHEMOTHERAPY

Result or Subgroup	Studies	Participants	Statistical Method	Estimated effect
Mortality in 12 months	3[6-8]	1,298	Difference in risk (M-H, Fixed, 95% CI)	-0.08 [-0.13, -0.04] NNT 12, 95% CI 7 to 25
Adverse events with a degree greater than or equal to 3	3[6-8]	1,286	Difference in risk (M-H, Fixed, 95% CI)	0.03 [-0.03, 0.08]

QUALITY OF EVIDENCE FOR THE DEATH OUTCOME

Pembrolizumab versus Chemotherapy

Question: Mortality with the use of pembrolizumab compared to chemotherapy for locally advanced or metastatic NSCLC.

TABLE 7. ANALYSIS OF THE QUALITY OF EVIDENCE (GRADE PRO SOFTWARE)[6]

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Pembrolizumab	Chemotherapy	Relative (95% CI)	Absolute (95% CI)		
Mortality in 12 months (follow-up: variation of 30 days to 12 months; assessed with: number of deaths in the intervention and control)												
2	randomized clinical trials	not severe	not severe	not severe	not severe	None	387/791 (48.9%)	438/788 (55.6%)	RR 0.88 (0.80 to 0.96)	minus 7 per 100 (from minus 11 to minus 2)	⊕⊕⊕⊕ HIGH	IMPORTANT

CI: Confidence interval; RR: Relative Risk

Pembrolizumab + Chemotherapy versus Chemotherapy

Question: Pembrolizumab + Chemotherapy compared to chemotherapy for locally advanced or metastatic NSCLC.

TABLE 8. ANALYSIS OF THE QUALITY OF EVIDENCE (GRADE PRO SOFTWARE)[6]

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistence	Indirect evidence	Imprecision	Other considerations	Pembrolizumab+ Chemotherapy	Chemotherapy	Relative (95% CI)	Absolute (95% CI)		
Mortality in 12 months (follow-up: variation of 30 days to 12 months; assessed with: number of deaths in the intervention and control)												
3	randomized clinical trials	not severe	not severe	not severe	not severe	None	490/748 (65.5%)	415/550 (75.5%)	RR 0.89 (0.83 to 0.95)	minus 8 per 100 (from minus 13 to minus 4)	⊕⊕⊕⊕ HIGH	IMPORTANT

CI: Confidence interval; RR: Relative Risk.

SYNTHESIS OF EVIDENCE

In adult patients with squamous or non-squamous NSCLC, locally advanced or metastatic, without previous systemic therapy, without mutations in the EGFR gene and gene rearrangement of the ALK, with a score of 0 or 1 in the Ecog performance scale, with at least one measurable lesion evaluated by Recist version 1.1, the treatment with pembrolizumab, associated or not to chemotherapy, compared with standard chemotherapy reduces the risk of death in 12 months by 7% and 8%, respectively. The quality of the evidence that supports this result is high.

For these patients, pembrolizumab reduced adverse events of degree ≥3 by 24% in comparison with chemotherapy, and when associated with it, it showed no difference in adverse events of degree ≥3 in comparison with chemotherapy alone, in up to 12 months. The quality of the evidence that supports this result is high.

RECOMMENDATION

The treatment with Pembrolizumab, associated or not with chemotherapy, in adult patients with squamous or non-squamous NSCLC, locally advanced or metastatic, without previous systemic therapy, without mutations in the EGFR gene and gene rearrangement of the ALK, with a score of 0 or 1 in the Ecog performance scale, with at least one measurable lesion evaluated by Recist version 1.1, and PD-L1 expression [Tumor Proportion Score (TPS)] ≥1% (positive PDL-1). High quality of evidence.

DISCUSSION

Among human neoplasms for which the investigation of PD-L1 immunohistochemical expression is validated by clinical studies, with an undeniable predictive value of the response to anti-PD-1 therapies available, the non-small cell lung carcinomas stand out. The PD-L1 expression on the tumor-cell membrane is indicated as an aid in the identification of patients for treatment with pembrolizumab. The result of the PD-L1 expression is determined by the percentage of tumor cells with membrane immunostaining (full or basolateral) of any intensity and/or by the percentage of immune cells associated with the tumor with immunostaining of any intensity, according to the type of neoplasia under analysis. Patients with a tumor proportion score (*TPS*) <1% of the PD-L1 biomarker in the tumor tissue are considered PD-L1 negative and with TPS ≥1% positive.

In the comparison pembrolizumab *versus* chemotherapy, we included two studies[1,7] that included only patients with TPS ≥1% (PDL-1 positive). However, in the comparison of pembrolizumab plus chemotherapy *versus* chemotherapy alone, the three studies assessed[3-5] included patients with TPS <1% (PDL-1 negative) and TPS ≥1% (1–49% and ≥50%), uniformly distributed between the groups, with an analysis of these subgroups.

It should be emphasized, however, that the greater the number of subgroups analyzed in one sample, the higher the probability of false-positive results; it is also added to that limitation the frequent tendency of

reporting, after various analyses, only the significant results, which can potentially distort the interpretation of the results. In the event of a positive result, is there effectively a significant difference in the treatment effect, or it is mere occurrence at random (considering the absence of a prior determination of sampling and subsequent statistical power for such difference)[3]?

Considering that: there is a lack of RCTs with a prior determination of sampling comparing positive and negative PDL-1 patients, and the mechanism of action of pembrolizumab in patients with NSCLC.

ANNEX I

The purpose of this assessment is to identify the benefits of immunotherapy with pembrolizumab, associated or not with chemotherapy, in the treatment of patients with locally advanced or metastatic NSCLC, without mutations of the EGFR gene or gene rearrangement of the *ALK,* with PD-L1 gene expression, in comparison to chemotherapy alone (current standard treatment).

Clinical question

What is the impact of pembrolizumab, associated or not to chemotherapy, on the outcomes of overall mortality (death from any cause) and adverse events in the treatment of patients with advanced NSCLC when compared to chemotherapy alone?

Structured question

Patient – Adult patients with advanced NSCLC;

Intervention – Treatment with Pembrolizumab alone or combined with chemotherapy;

Comparison – Chemotherapy, except monochemotherapy with docetaxel;

Outcomes – Death (due to any cause) and adverse events.

Eligibility criteria

Excluded the outcomes – quality of life, objective response, survival free of progression – analyses of tumor proportion score (*TPS*) of the PD-L1 biomarker on the tumor tissue were not evaluated.

Randomized clinical trial.

Without time or language restrictions.

Full text available for access.

Search strategies used

The search for evidence will be conducted on Medline virtual scientific information database, using the following search strategy:

(((((Pulmonary Neoplasms OR Lung Neoplasm OR Pulmonary Neoplasm OR Lung Neoplasms OR Lung Cancers OR Lung Cancer OR Lung Neoplasm OR Lung Neoplasms OR PD-L1))) AND ((pembrolizumab) AND Random*))) OR ((((Pulmonary Neoplasms OR Lung Neoplasm OR Pulmonary Neoplasm OR Lung Neoplasms OR Lung Cancers OR Lung Cancer OR Lung Neoplasm OR Lung Neoplasms))) AND ((pembrolizumab OR PD-L1) AND Random*)).

In the Central/Cochrane databases, the search strategy will be: (Lung Neoplasm OR Pulmonary Neoplasm OR Lung Cancer) AND pembrolizumab.

Extraction of results

We will extract the following data from the studies: name of the author and year of publication, study population, intervention and comparison methods, the absolute number of deaths and adverse events, time of follow-up.

Randomized clinical trials will have their risk of biases analyzed according to the following criteria: randomization, blinded allocation, double-blinding, losses, prognostic characteristics, presence of relevant outcome, time for the outcome, method for outcome measurement, sample size calculation, early interruption, presence of other biases.

The results will be presented as the difference in the risk of death or adverse events between pembrolizumab therapy alone or in combination with chemotherapy, and the treatment with chemotherapy alone. The confidence level adopted was 95%.

The results of the studies included will be combined and meta-analyzed by RevMan 5.3[8], and the difference in overall risk will be the final measure used to support the synthesis of evidence that will answer the clinical question of this review.

Furthermore, the quality of evidence will be graded as high, moderate, low, or very low using the Grade instrument[6] and taking into account the risk of bias, the presence of inconsistency, vagueness or indirect evidence in the meta-analysis of the outcomes of death and adverse events, and the presence of publication bias. Table 5. Quality of evidence (GRADE).

Explanation: a. 50% with randomization inadequate or not described; only one with blinded allocation,

TABLE 5. QUALITY OF EVIDENCE (GRADE).

Evaluation of certainty							Nº of patients		Effect		Certainty	Importance
Nº of studies	Design of the study	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Levosimendan	Dobutamine	Relative (95% CI)	Absolute (95% CI)		
Mortality at 24 hours (assessed with: Number of deaths in 24 hours)												
4	randomized clinical trials	severe [a]	not severe	not severe	not severe [b]	none	1/208 (0.5%)	4/204 (2.0%)	RR 3.05 (0.49 to 18.90)	40 more per 1,000 (from minus 10 to 351 more)	⊕⊕⊕◯ MODERATE	IMPORTANT
Mortality at 30 days (assessed with: Number of deaths up to 30 days)												
3	randomized clinical trials	not severe	severe [c]	not severe	severe [d]	none	93/836 (11.1%)	125/831 (15.0%)	RR 1.74 (0.94 to 3.19)	111 more per 1,000 (from minus 9 to 329 more)	⊕⊕◯◯ LOW	IMPORTANT
Mortality between 120 and 180 days (follow-up: variation 120 days to 180 days; assessed with: Number of deaths in this period)												
4	randomized clinical trials	severe [e]	not severe	not severe	severe [f]	none	206/811 (25.4%)	236/807 (29.2%)	RR 1.15 (0.98 to 1.35)	44 more per 1,000 (from minus 6 to 102 more)	⊕⊕◯◯ LOW	IMPORTANT

CI: Confidence interval; RR: Relative Risk

three studies without blinding; only with sample size calculation; b. The overall result does not exclude the benefit or harm; c. Test for inconsistency I2 equal to 70%; small overlap of confidence intervals; d. The result does not exclude great harm or benefit; e. 2 studies with Jadad = 1 and 2 studies with Jadad >= 3; f. The result does not exclude great harm or benefit

Critical evaluation and level of evidence

All evidence retrieved was evaluated according to their risk of bias (level of error estimated and inherent in the delineation of research or study design used).

For the evaluation of the risk of bias of the RCTs, the following items were considered: if the issue was focal, appropriate randomization, blinded allocation, double-blind, losses (>20%), prognostic characteristics, outcomes (time, adequacy, measurement), analysis by intention to treat (ITT), sample size calculation, Jadad scale[7].

Reflecting the level of uncertainty of the results (effects of benefit or harm), the strength of the evidence can be very low, low, moderate, or strong, depending on:

1. The risks of bias of each individual and meta-analyzed study.

2. The magnitude and accuracy of the results for each outcome analyzed.

3. The relevance, applicability, and generalization of these results.

Presentation of the results

In the search for evidence, we retrieved 244 papers, selecting by title and abstract nine studies of immunotherapy with pembrolizumab, associated or not to chemotherapy, in the treatment of patients with NSCLC, compared with chemotherapy alone, of which six studies were accessed since they met the eligibility criteria for analysis of the full text. Of the six studies, five were selected to support this assessment[1-5]; the grounds for exclusion and the list of studies excluded are available in the references, Figure 6 and Table 9 in the Annexes.

NSCLC = Non-small-cell lung carcinoma; CNS = Central nervous system; AUC = Area under the concentration-time curve; OS = overall survival; SFP = Survival free of progression; PD-L1 = Programmed death ligand 1; TPS = Tumor proportion score of biomarker PD-L1 in tumor tissue; EGFR = Epidermal growth factor receptor; ALK = Anaplastic Lymphoma Kinase; Ecog = Eastern Cooperative Oncology Group; Recist = Response Evaluation Criteria in solid tumors.

TABLE 2. DESCRIPTION OF THE BIASES IN THE STUDIES INCLUDED

Study and year	Random	Blinded allocation	Dou-ble-blind	Losses	Charac-teristics Prognosis	Out-comes	Analysis per ITT	Sample size cal-culation	Jadad[7]
Mok TSK 2019									4
Reck M 2016									4
Gandhi L 2018									5
Paz-Ares L 2018									5
Langer CJ 2016									4

ITT = intention-to-treat analysis Low risk of bias the Presence of bias Unclear risk of bias

TABLE 1. DESCRIPTION OF THE STUDIES INCLUDED

STUDY	Population	Intervention	Comparison	Out-comes	Time (median, months)
Mok TSK, et al. 2019 Keynote-042	≥18 years) with locally advanced or metastatic NSCLC, not previously treated, without EGFR mutation or ALK translocation, and with a score of 0 or 1 in the Ecog performance scale, life expectancy of three months or more, and a PD-L1 TPS of 1% or greater. Excluded if: unstable or untreated CNS metastases, a history of noninfectious pneumonitis requiring systemic glucocorticoids, active autoimmune disease, systemic immunosuppressive treatment, or active infection by the virus of hepatitis B or C.	Pembrolizumab 200 mg every three weeks, in up to 35 cycles	Carboplatin to achieve an AUC of 5-6 mg/mL per minute plus paclitaxel 200 mg/m^2 or pemetrexed 500 mg/m^2	OS SFP Adverse events	12.8
Reck M, et al. 2016 Keynote-024	≥18 years, NSCLC histologically or cytologically confirmed in stage IV, without EGFR mutations or ALKtranslocation, without prior systemic therapy for metastatic disease, with a 0 or 1 score in the ECOG performance status, at least one measurable lesion according to Recist version 1.1, life expectancy of at least three months, and a 50% or more score of PD-L1 tumor proportion. Excluded if: receiving systemic glucocorticoids (excluding daily replacement therapy with glucocorticoids for conditions such as pituitary or adrenal insufficiency) or other immunosuppressive treatment, with untreated brain metastases, active autoimmune disease for which they received systemic treatment over the past two years, active interstitial lung disease or pneumonitis history for which they received glucocorticoids.	Pembrolizumab in a fixed dose of 200 mg every three weeks.	Platinum-based chemotherapy to the choice of the researcher during 4 to 6 cycles: carboplatin plus pemetrexed, cisplatin plus pemetrexed, carboplatin plus gemcitabine, cisplatin plus gemcitabine, cisplatin plus gemcitabine or carboplatin plus paclitaxel. The chemotherapy regimens that included pemetrexed were allowed only for patients who had non-squamous tumors. Crossover allowed.	OS SFP Adverse events	25.2
Gandhi L et al. 2018 Keynote-189	≥18 years, metastatic NSCLC, non-squamous, without mutations in the EGFR gene and ALK rearrangement, without prior therapy for the disease. Other conditions: an ECOG performance status of 0 or 1; at least one measurable lesion according to Recist version 1.1, and provide a sample of the tumor for determining the PD-L1state. Excluded if: symptomatic CNS metastases, history of non-infectious pneumonitis requiring the use of glucocorticoids, autoimmune disease with systemic therapy in the past two years, medical condition requiring immunosuppression, received more than 30 Gy of radiotherapy to the chest over the past six months.	Pemetrexed (500 mg/m^2 IV) and a platinum-based drug at the choice of the investigator (cisplatin 75 mg/m^2 of body surface or carboplatin AUC 5mg/mL/min intravenously) plus 200 mg of pembrolizumab every three weeks, for four cycles, followed by pembrolizumab until a total of 35 cycles, plus maintenance therapy with pemetrexed.	Pemetrexed (500 mg/m^2 IV) and a platinum-based drug at the choice of the investigator (cisplatin 75 mg/m^2 of body surface or carboplatin AUC 5mg/mL/min intravenous) plus a placebo every three weeks, for four cycles, followed by a placebo until a total of 35 cycles, plus maintenance therapy with pemetrexed.	OS SFP. Adverse events	10.5

STUDY	Population	Intervention	Comparison	Out-comes	Time (median, months)
Paz-Ares L et al. 2018 Keynote-407	≥18 years; squamous NSCLC stage IV, without prior systemic therapy for metastatic disease, ECOG performance status score of 0 or 1, with at least one measurable lesion according to Recist version 1.1, provided a tumor sample for determining the PD-L1 state. Excluded if: symptomatic metastases of the CNS, history of non-infectious pneumonitis that required the use of glucocorticoids; autoimmune disease that required systemic therapy in the past two years; medical condition requiring immunosuppression.	Pembrolizumab 200 mg for up to 35 cycles + carboplatin and paclitaxel or nanoparticle albumin-bound [NAB] - paclitaxel for the first four cycles.	Saline (placebo) for up to 35 cycles + carboplatin and paclitaxel or nanopartícula albumin-bound [NAB] - paclitaxel during the first four cycles.	SFP OS Adverse events	7.8
Langer CJ et al. 2016 Keynote-021	No previous systemic treatment for non-squamous NSCLC, cytologically or histologically confirmed, Stage IIIB or IV, and absence of EGFR target mutations or ALK translocations; ECOG performance status of 0 or 1, at least one measurable lesion evaluated by Recist version 1.1, life expectancy of three months or more, and supply of a sample of the tumor for evaluation of the PD-L1expression. Excluded if: received more than 30 Gy of radiation to the lungs in the previous six months; continuous use of systemic corticosteroids or other immunosuppressive treatment, active autoimmune disease that required systemic treatment in the past two years (excluding replacement therapy), untreated brain metastases (allowed treated, stable metastasis), or active interstitial lung disease, history of pneumonitis requiring intravenous glucocorticoids.	Pembrolizumab 200 mg + carboplatin AUC 5 mg/mL/min and pemetrexed intravenous 500 mg/m^2 every three weeks followed by pembrolizumab for 24 months and maintenance therapy with undefined pemetrexed.	Four cycles of carboplatin and pemetrexed, followed by maintenance therapy with undefined pemetrexed.	Objective Response SFP OS Adverse events	23.9

FIGURE 6. FLOWCHART

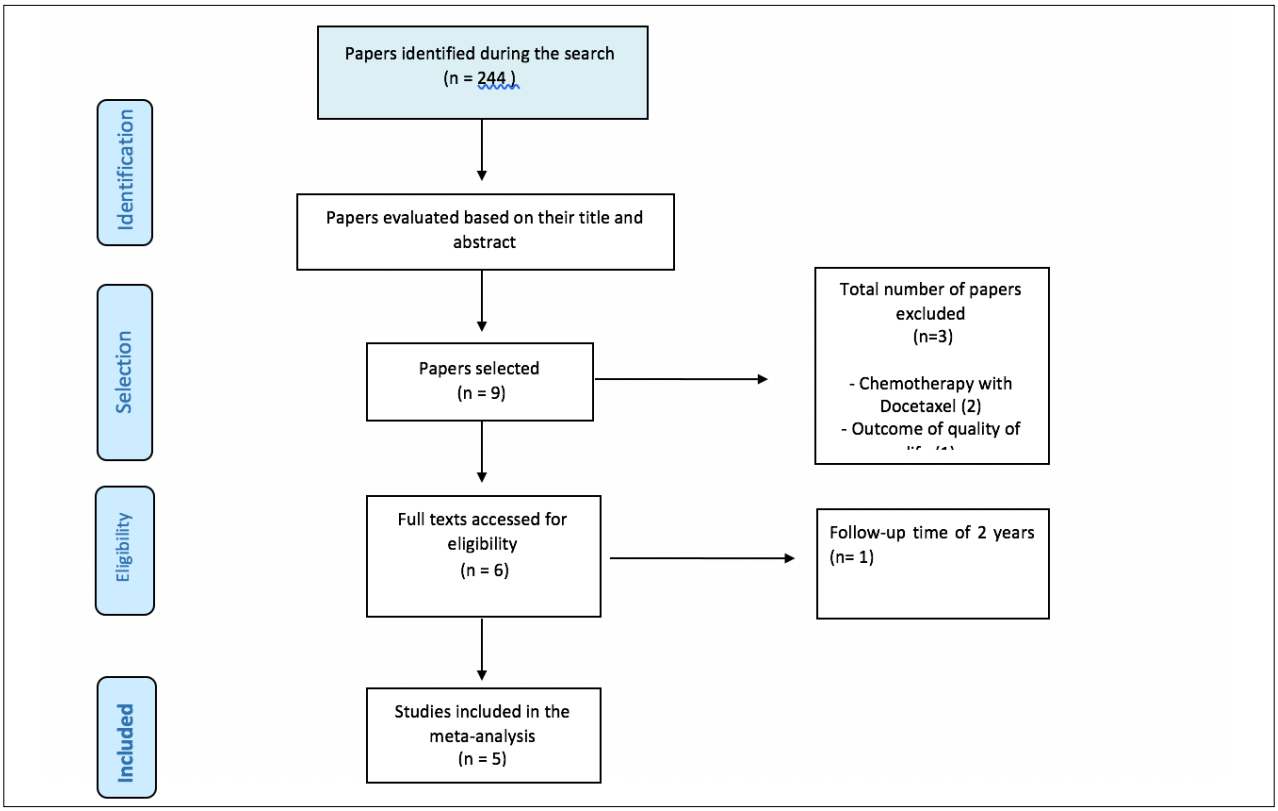


TABLE 9. STUDIES EXCLUDED AND REASON FOR EXCLUSION

Study	Reason for exclusion
Barlesi F, et al. 2019	Docetaxel intervention and quality of life outcome
Reck M, et al. 2019	Follow-up time of 2 years
Brahmer JR, et al. 2017	Outcome of quality of life
Herbst RS, et al. 2016	Docetaxel intervention

Application of evidence - Recommendation

The available evidence will follow some principles of exposure– it will be by outcome and will have as components: number of patients, type of comparison, magnitude, and precision (standard deviation and 95% CI).

Its strength will be estimated (Oxford[16]/Grade[8]) as 1b and 1c (grade A) or strong, and as 2a, 2b and 2c (grade B) or moderate weak, or very weak.

Conflict of interest

There is no conflict of interest related to this review that can be declared by any of the authors.

Final declaration

The Guidelines Project, an initiative of the Brazilian Medical Association in partnership with the Specialty Societies, aims to reconcile medical information in order to standardize approaches that can aid the physician's reasoning and decision-making process. The information contained in this project must be submitted to the evaluation and criticism of the physician responsible for the conduct to be followed, given the reality and clinical condition of each patient.

REFERENCES

1. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomized, open-label, controlled, phase 3 trial. Lancet 2019 4;393:1819-1830. PMID: 30955977
2. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016 10;375:1823-1833. PMID: 27718847
3. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomized, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol 2016;17:1497-1508. PMID: 27745820
4. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med 2018 31;378:2078-2092. PMID: 29658856
5. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, et al. Pembrolizumab plus Chemotherapy for Squamous Non-Small-Cell Lung Cancer. N Engl J Med 2018 22;379:2040-2051. PMID: 30280635
6. GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University, 2015 (developed by Evidence Prime, Inc.). Available from gradepro.org.
7. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996; 17:1-12.
8. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.
9. Oxman A, Guyatt G. When to believe a subgroup analysis. In: Guyatt G. User's guides to medical literature. A manual of evidence based clinical pratice. New York: Jama Press, 2003.

REFERENCES ANNEXES

1. Barlesi F, Garon EB, Kim DW, Felip E, Han JY, Kim JH, et al. Health-Related Quality of Life in KEYNOTE-010: a Phase II/III Study of Pembrolizumab Versus Docetaxel in Patients With Previously Treated Advanced, Programmed Death Ligand 1-Expressing NSCLC. J Thorac Oncol 2019;14:793-801. PMID: 30711649
2. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. J Clin Oncol 2019 1;37:537-546. PMID: 30620668
3. Brahmer JR, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, et al. Health-related quality-of-life results for pembrolizumab versus chemotherapy in advanced, PD-L1-positive NSCLC (KEYNOTE-024): a multicentre, international, randomised, open-label phase 3 trial. Lancet Oncol 2017;18:1600-1609. PMID: 29129441
4. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016 9;387:1540-50. PMID: 26712084

Elderly patients with glioblastoma: the impact of the surgical resection extent on survival

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Question: What are the results (overall survival time) according to the tumor resection extent?

Answer: The mean combined overall survival time (COS) was 13.13 months in patients in whom total resection was possible, 7.52 months in those who underwent partial resection, and 2.56 months in patients who underwent biopsy alone.[1]

REFERENCE

1. Cunha M, Esmeraldo A, Henriques L, Santos M, Medeiros R, Botelho R. Elderly patients with glioblastoma: the impact of surgical resection extent on survival. Rev Assoc Med Bras. 2019; 65(7):937-945

Autoimmune Polyglandular Syndrome type 2

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SUMMARY

Autoimmune polyglandular syndrome type 2 (APS 2) is defined by the presence of Addison's disease (AD) associated with autoimmune thyroid disease and/or Type 1 diabetes mellitus (T1DM). It is a rare disease, affecting about 1.4-2 cases/100,000 inhabitants. Its less frequent clinical presentation is the combination of AD, Graves' disease, and T1DM. We present the case of a 42-year-old woman with a history of total thyroidectomy due to Graves' disease, type 2 diabetes mellitus, and hypertension, who sought the ED due to asthenia, dizziness, nausea, and vomiting. She reported having stopped antihypertensive therapy due to hypotension and presented a glycemic record with frequent hypoglycemia. On physical examination, she had cutaneous hyperpigmentation. She had no leukocytosis, anemia, hypoglycemia, hyponatremia or hyperkalemia, and a negative PCR. Serum cortisol <0.5 ug/dl (4,3-22,4), urine free cortisol 9 ug/24h (28-214), ACTH 1384 pg/mL (4,7-48,8), aldosterone and renin in erect position of 0 pg/ml (41-323) and 430.7 uUI/ml (4.4-46.1) respectively. Quantiferon TB was negative; computerized axial tomography of the adrenals showed no infiltrations, hemorrhage, or masses. The 21-hydroxylase antibody assay was positive. B12 vitamin was normal, anti-GAD antibodies were positive, anti-insulin, anti-IA2, and anti-transglutaminase antibodies were all negative. The patient started insulin therapy and treatment for AD with prednisolone and fludrocortisone with good clinical response. This case aims to alert to the need for high clinical suspicion in the diagnosis of AD. Since this is a rare autoimmune disease, it is important to screen for other autoimmune diseases in order to exclude APS.

KEYWORDS: *Polyendocrinopathies, Autoimmune. Addison's Disease. Diabetes Mellitus, Type 1. Thyroid Diseases*

INTRODUCTION

Autoimmune polyglandular syndromes are a rare group of polyendocrine conditions that included multiple glandular deficiencies associated with other autoimmune diseases[1], such as hypergonadotropic hypogonadism, vitiligo, chronic atrophic gastritis, pernicious anemia, chronic autoimmune hepatitis, and celiac disease. Autoimmune polyendocrine syndrome type 2 (APS II) is defined by the presence of Addison's disease (AD) associated with autoimmune thyroid disease and/or diabetes mellitus (DM) type 1[2]. It is a rare condition, affecting approximately 1.4-2 cases/100,000 inhabitants[3]. Its least frequent clinical presentation is the combination of Graves' disease and diabetes mellitus type 1. We present a case of APS II with the complete triad.

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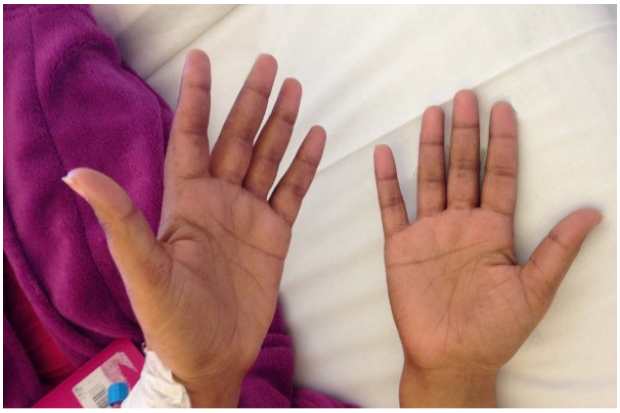
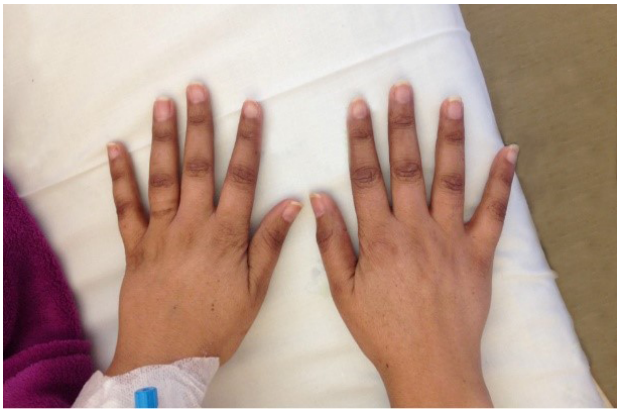
CLINICAL CASE

We present a 42-year-old woman, who was emigrated from France between age 19 and 36, with a history of total thyroidectomy due to Graves' disease 11 years ago, DM type 2 with four years of evolution and hypertension, who sought the DE due to a continued condition, with three months of evolution, asthenia, weight loss (12 kg in three months), dizziness, abdominal pain predominantly in the right quadrants, nausea, and vomiting. She reported having suspended anti-hypertension therapy due to hypotension and presented a glycemic record with frequent hypoglycemia. Other medical history included dyslipidemia, asthma, and repeated urinary tract infections (ITUs). She was medicated with 100 mg sitagliptin, 5 mg folic acid, 0.1 mg levothyroxine, 10 mg atorvastatin, 320 µg budesonide+formoterol + 9 µg SOS. She had complementary diagnostic exams from a DE context, from 1.5 months before, due to a similar clinical scenario: analytical assay with slight microcytic anemia, negative CRP (Table 1), abdominal/pelvic/kidney/bladder ultrasound and combur without abnormalities. She was discharged with an indication for symptomatic treatment and an iron kinetics analysis, video colonoscopy, and outpatient EGD. The iron kinetics and video colonoscopy showed no abnormalities. The EGD was still pending completion. Upon physical examination at DE, TA was 104/77 mmHg, without orthostatic hypotension, FC: 83/min, no fever, discolored mucosa, with skin hyperpigmentation (Figures 1A and 1B); the rest of the exam showed no other abnormalities. After suspected suprarenal insufficiency, the examination proceeded. Analytically, there was no leukocytosis, anemia, hypoglycemia, hyponatremia, or hyperkalemia, and CRP was negative. Morning serum cortisol <0.5 ug/dl (4.3-22.4), free cortisol in urine 9 ug/24h (28-214), ACTH 1384 pg/mL (4.7-48.8), aldosterone and renin in an upright position of 0 pg/mL (41-323) and 430.7 IU/mL (4.4-46.1), respectively (Table 1). An additional study was conducted to investigate the cause of primary suprarenal insufficiency. Negative Quantiferon TB, suprarenal computed axial tomography without infiltrations, hemorrhage, or masses. Results for 21-hydroxylase antibodies were positive. After the autoimmune cause was confirmed and with a previous history of autoimmune thyroid disease, the investigation continued with normal vitamin B 12, positive anti-GAD, and negative anti-insulin, anti-IA2, anti-transglutaminase. In this context,

TABLE 1. ANALYTICAL STUDY.

Test	Results 1.5 months before	Results in hospitalization	Reference values
Leukocytes	4.8x10^9/L	5.0x10^9/L	4.5 – 11.50
Hemoglobin	11.4 g/dL	13.4 g/dL	12 – 15
Glucose	171 mg/dL	154 mg/dL	74 - 106
Sodium	134 mEq/L	136 mEq/L	136 - 145
Potassium	4.3 mEq/L	4.7 mEq/L	3.4 – 4.4
CRP	0.09 mg/dL	1 mg/dL	<0.50
AST	56 UI/L		3 – 31
ALT	61 UI/L		3 – 31
Urea	26 mg/dL	47 mg/dL	16 – 42
Creatinine clearance	0.5 mg/dL	0.7 mg/dL	0.5 – 1.2
Urinary cortisol		9 ug/24h	28-214
Morning serum cortisol		<0.5 ug/dl	4.3-22.4
ACTH		1.384 pg/mL	4.7-48.8
Renin		430.7 UI/mL	4.4-46.1
Aldosterone		0 pg/mL	41-323





FIGURES 1A AND 1B. SKIN HYPERPIGMENTATION.

the patient started insulin therapy and targeted treatment to AD with hydrocortisone and fludrocortisone, with good clinical response, maintaining a follow-up in external consultations.

DISCUSSION

There are three types of APS, of which SPGA2 is the most common and most frequent in women between the third and fourth decades of life.

Our patient was a middle-aged woman, precisely at the peak of APS II incidence. The coexistence of Graves' disease, DM type 1, and AD is in line with the APS II diagnosis.

Approximately 50% of patients with suprarenal insufficiency have other autoimmune diseases associated with it, and thyroid disease is the most frequent of them — however, only 1% of patients with autoimmune thyroid disease suprarenal insufficiency[4,5].

Suprarenal insufficiency is the first manifestation in 50% of cases. It appears simultaneously with diabetes mellitus or autoimmune thyroid disease in 20% of cases, and after these pathologies in approximately 30% [6,7].

In this case, the first manifestation was the thyroid disease, with adrenal insufficiency emerging 11 years later.

Since this was a patient with a history of recurrent UTIs, complaints of abdominal pain, nausea, and vomiting, in the first approach at the DE exams were conducted in order to exclude acute pathologies, such as UTIs, acute cholecystitis/cholangitis, renal colic, and adnexal pathology.

Since there was a nonspecific and continued clinical presentation of anorexia with weight loss, nausea, and vomiting associated with mild microcytic anemia, an outpatient examination was conducted in order to exclude gastrointestinal pathologies, in particular, neoplastic disease.

The early diagnosis of AD significantly reduces the morbidity and mortality of the disease. However, since the clinical presentation can by inaccurate and nonspecific, in most cases, the diagnosis is delayed. The concept of APS consists in the fact that a patient with autoimmune disease has a higher probability of developing a new autoimmune disease than the general population. It is described that the circulating autoantibodies associated with a particular type of disease may be present months to years prior to its development[5]. Thus, after the diagnosis of an autoimmune disease, it is crucial to screen for other associated pathologies. In this patient, the fact that she was emigrated without a medical follow-up may have influenced her medical guidance and the late diagnosis of Lada DM and AD.

CONCLUSION

This case report aims to alert to a rare entity (AD), with a not very specific clinical presentation, that requires a high degree of suspicion to reach an early diagnosis, reinforcing the idea that it is necessary to make the appropriate screening for other associated autoimmune diseases.

Contribution of the authors

All authors contributed equally to the development of this work.

RESUMO

A síndrome poliglandular autoimune tipo 2 (SPGA2) é definida pela presença de doença de Addison (DA) associada à doença tiroideia autoimune e/ou diabetes mellitus tipo 1 (DMT1). Trata-se de uma doença rara, afetando cerca de 1,4-2 casos/100.000 habitantes. A apresentação clínica menos frequente é a combinação de DA, doença de Graves e DMT1.

Apresenta-se mulher de 42 anos, com antecedentes de tiroidectomia total por doença de Graves, diabetes mellitus tipo 2 e hipertensão, que recorre ao SU por quadro arrastado de astenia, emagrecimento, tonturas, náuseas e vômitos. Referia ter suspendido terapêutica anti-hipertensora por hipotensão e apresentava registro glicêmico com hipoglicemias frequentes. Ao exame físico, salientava hiperpigmentação cutânea. Analiticamente sem leucocitose, anemia, hipoglicemia, hiponatremia ou hipercaliemia, PCR negativa. Cortisol sérico matinal <0,5 ug/dl (4,3-22,4), cortisol livre na urina 9 ug/24h (28-214), ACTH 1.384 pg/mL (4,7-48,8), aldosterona e renina em posição ereta de 0 pg/mL (41-323) e 430,7 uUI/mL (4,4-46,1), respectivamente. Realizado estudo complementar para averiguar causa de insuficiência suprarrenal primária. Quantiferon TB negativo, tomografia axial computadorizada das suprarrenais sem infiltrações, hemorragia ou massas. Anticorpos anti-21-hidroxilase positivos. Foi aprofundada a investigação com vitamina B12 normal, anti-GAD positivo, anti-insulina, anti-IA2, antitransglutaminase, negativos. Nesse contexto, a doente iniciou insulinoterapia e tratamento dirigido para a DA com prednisolona e fludrocortisona, com boa resposta clínica.

Este caso tem como objetivo alertar para a necessidade de elevada suspeição clínica no diagnóstico de DA. Sendo esta uma doença autoimune rara, é importante rastrear outras doenças autoimunes no sentido de excluir SPGA.

PALAVRAS-CHAVE: *Poliendocrinopatias autoimunes. Doença de Addison. Diabetes mellitus tipo 1. Doenças da glândula tireoide.*

REFERENCES

1. Dittmar M, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. J Clin Endocrinol Metab. 2003;88(7):2983-92.
2. Eisenbarth GS. Autoimmune polyendocrine syndromes. Adv Exp Med Biol. 2004;552:204-18.
3. Gupta AN, Nagri SK. Schmidt's syndrome: case report. Australas Med J. 2012;5(6):292-5.
4. Betterle C, Volpato M, Rees Smith B, Furmaniak J, Chen S, Greggio NA, et al. I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. J Clin Endocrinol Metab. 1997;82(3):932-8.
5. Michels AW, Eisenbarth GS. Autoimmune polyendocrine syndrome type 1 (APS-1) as a model for understanding autoimmune polyendocrine syndrome type 2 (APS-2). J Intern Med. 2009;265(5):530-40.
6. Spinner MW, Blizzard RM, Childs B. Clinical and genetic heterogeneity in idiopathic Addison's disease and hypoparathyroidism. J Clin Endocrinol Metab. 1968;28(6):795-804.
7. Nerup J. Addison's disease: clinical studies. A report fo 108 cases. Acta Endocrinol (Copenh). 1974;76(1):127-41.

Artificial intelligence in the diagnosis of cardiovascular disease

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SUMMARY

Artificial intelligence (AI) is a field of computer science that aims to mimic human thought processes. AI techniques have been applied in cardiovascular medicine to explore novel genotypes and phenotypes in existing diseases, improve the quality of patient care, enabling cost-effectiveness, and reducing readmission and mortality rates. The potential of AI in cardiovascular medicine is tremendous; however, ignorance of the challenges may overshadow its potential clinical impact. This paper gives a glimpse of AI's application in cardiovascular clinical care and discusses its potential role in facilitating precision cardiovascular medicine.

KEYWORDS: *Machine learning. Artificial Intelligence. Algorithms.*

INTRODUCTION

Artificial intelligence (AI) is a field of computer science that seeks to mimic the human thought processes, learning ability, and the storage of knowledge. In the 21st century, the paradigm is shifting from the use of traditional statistical tools to AI in cardiovascular (CV) medicine to allow for better accuracy.

Big Data

There are a series of demographic data (such as electronic records and standardized platforms) available today that alone bring no benefits, but, when processed by AI, these *Big Data* improve the practice of clinical care. Big Data analysis by AI can predict the identification of new phenotypes, such as of heterogeneous syndromes, support clinical decisions such as the selection of anticoagulant agents in patients with nonvalvular atrial fibrillation, and assist in the identification of unknown risk factors, such as in acute coronary syndrome. With that, the availability of automated tools for real-time decision support using AI in standardized electronic records also grows[1,2].

Machine Learning

Machine learning is a subdiscipline of AI and can be categorized into three types of learning, i.e., supervised, unsupervised, and by reinforcement. The learning curves and the area under the curve (AUC) are important considerations when choosing a machine learning algorithm, while C-statistic is important in the choice of traditional methods of data processing. As in traditional statistics, machine learning requires a sufficient data set for training (the sample size in traditional statistics), and there should be no lack of adjustments, i.e., underfitting and overfitting (and alfa should not be greater than 0.05 in traditional statistics).

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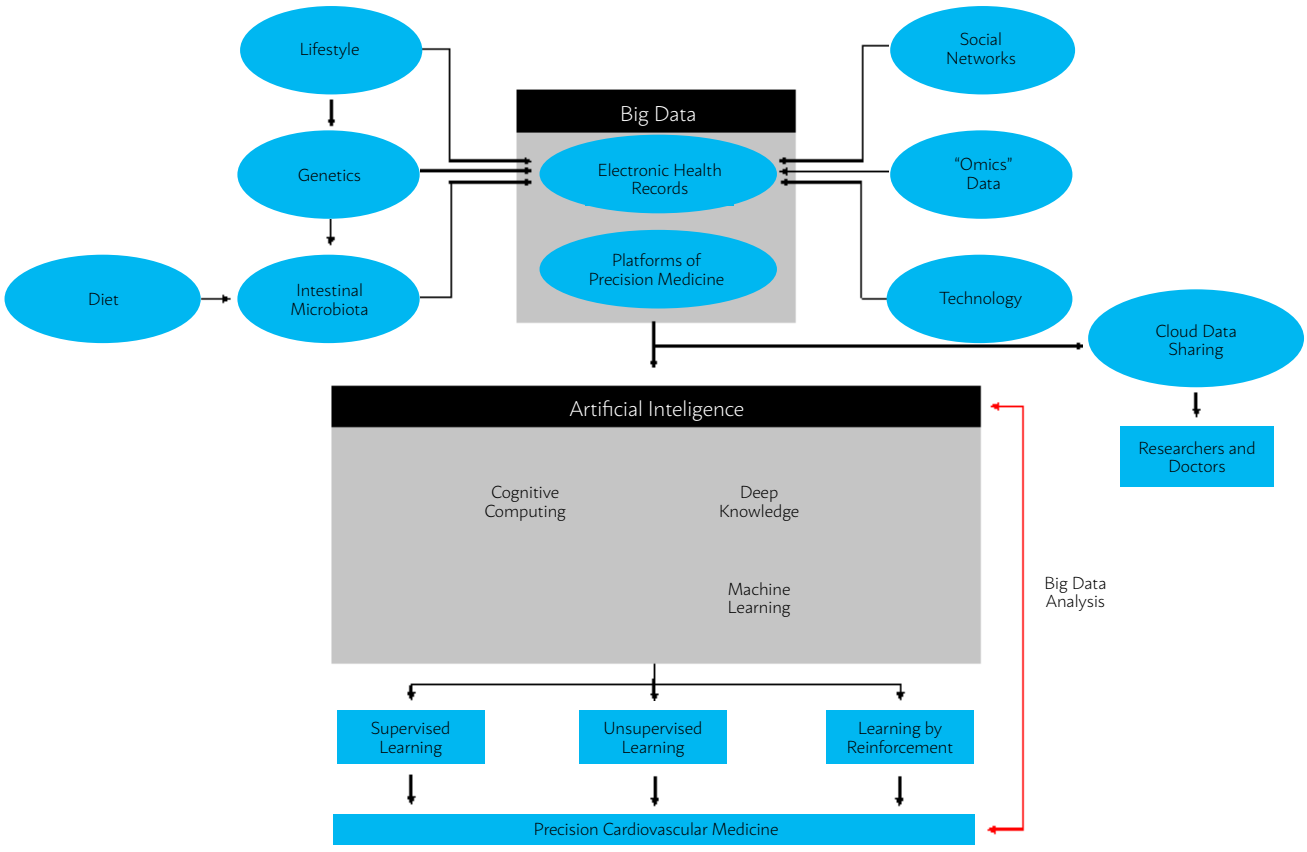
Supervised Learning

Supervised learning has algorithms that use a data set labeled by humans to predict the desired and known outcome; these are successfully applied in the prediction, diagnosis, and treatment of CV diseases, as well as in the analysis of CV images. Such learning is great for classification and regression. The algorithms that can be used include artificial neural networks (ANN), support vector machine (SVM), random forests, decision tree, naive Bayes classifier, fuzzy logic, and K-nearest neighbor (KNN). The selection variables of a particular algorithm include the characteristic of the data and training time. ANN and SVM are the most popular types because they are compatible with "omics" data (genomic, metabolomic, and proteomic). ANN and SVM feature good performance in the classification of text in electronic health records[3]. ANN algorithms mimic the human neurons and present good results for processing electrocardiogram (ECG) data and can be used on deep knowledge[4,5]. The *random* forests decision tree and Naive Bayes classifier algorithms are less accurate than ANN and SVM but are easier to be used and require fewer data. Random forests present each independently trained forest and have been used in coronary computed tomography angiography[6,7]. The system of the decision tree is easy to understand, unlikely to find overfitting, and used to predict CV risk[8]. The naive Bayes classifier is a simple classifier derived from Bayes theorem and can be used in problems of text classification, in the identification of CV risk factors[9]. Fuzzy logic is similar to human reasoning, in which the logic returns values (for example, 30% of probability of acute myocardial infarction)[10]. KNN runs quickly on small training data sets and can be used to interpret ECGs but requires more space

FIGURE 1. ILLUSTRATION OF ARTIFICIAL INTELLIGENCE IN THE DIAGNOSIS OF CARDIOVASCULAR DISEASE



Adapted from Krittanawong, C. et al. J Am Coll Cardiol. 2017;69(21):2657-64.

Big Data (Genetics, Social Networks, Environment, and lifestyle-related factors, or 'omics' data") can be stored in Electronic Health Records or Platforms of Precision Medicine and can be shared on clouds with other researchers or doctors for data analysis using secure technology systems. Big Data analysis using artificial intelligence (Machine Learning, Deep Learning, or Cognitive Computing) and the three main types of learning algorithms (Supervised, Unsupervised, and Reinforcement) will allow for Precision Cardiovascular Medicine.

for the large amount of data[11]. In supervised learning, small training data sets can lead to inaccurate decisions in the data sets test if the training data set is skewed.

Unsupervised Learning

Unsupervised learning is based on finding hidden patterns in data without human feedback. This type of learning is often used in deep knowledge and can allow for the identification of new phenotypes of cardiomyopathy and be used on blood bank platforms to identify hypertension genotypes. The algorithms used can be classified into clustering or association rule algorithms. Clustering algorithms can be used to group unlabeled data. Algorithms for association-rule learning help discover relationships between data items that are apparently unrelated (for example, 70% of patients who have had angioedema with angiotensin receptor-neprilysin inhibitors). Since the final cluster pattern depends on the initial cluster, one limitation of this learning process is the difficulty in identifying the pattern of the initial cluster.

Learning by Reinforcement

Learning by reinforcement can be seen as a hybrid of supervised and unsupervised learning. The objective of this type of learning is to maximize the accuracy of the algorithms by trial and error.

Deep Knowledge

Deep knowledge mimics the functioning of the human brain by using multiple layers of artificial neural networks that can generate automatic forecasts from a training data set. This knowledge can be widely used to recognize images (cardiac angiography and magnetic resonance)[12]. It can also be trained to perform an unsupervised learning task, such as a drug-drug interaction. Furthermore, there is no limitation regarding working memory. Deep knowledge has proved to be superior to other techniques of machine learning, such as SVM, because it can use multiple layers and transformations, in comparison to the two layers of MVR. Since deep knowledge is usually a non-linear analysis with many parameters and multiple layers, overfitting may be large, leading to a weak predictive performance[13,14].

Cognitive Computing

Cognitive Computing involves a self-learning system that uses machine learning, pattern recognition, and natural language processing to mimic the functioning of the human thought processes (Figure 1). A machine algorithm of cognitive learning, the classifier of associative memory (accuracy: 93.7%; AUC: 96.2%) used to classify constrictive pericarditis of restrictive cardiomyopathy for automated interpretation of data from tracking echocardiography of stains has proved to be superior than the random forest (accuracy: 88.3%; AUC: 94.2%) and SVM (accuracy: 87.4%; AUC: 92.2%)[15].

CONCLUSION

Over the past decade, several machine learning techniques have been used to diagnose and predict cardiovascular diseases. Each problem requires some degree of understanding, regarding CV medicine and statistics, to apply the optimal algorithm for machine learning. In the near future, AI will cause a paradigm shift toward precision cardiovascular medicine.

Contribution of the authors:

All authors contributed equally to the development of this work.

RESUMO

A inteligência artificial (IA) é um campo da ciência da computação que tem como objetivo imitar os processos de pensamento humano. Técnicas de IA têm sido aplicadas na medicina cardiovascular para explorar novos genótipos e fenótipos em doenças existentes, melhorar a qualidade do atendimento ao paciente, possibilitar custo-efetividade e reduzir taxas de readmissão e mortalidade. Existe um grande potencial da IA na medicina cardiovascular; no entanto, a ignorância dos desafios pode ofuscar seu impacto clínico. Esse artigo fornece a aplicação da IA no atendimento clínico cardiovascular e discute seu papel potencial na facilitação da medicina cardiovascular de precisão.

PALAVRAS-CHAVE: *Aprendizado de máquina. Inteligência artificial. Algoritmos.*

REFERENCES

1. Harrell FE Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361-87.
2. van den Ham HA, Klungel OH, Singer DE, Leufkens HG, van Staa TP. Comparative performance of ATRIA, CHADS2, and CHA2DS2-VASc risk scores predicting stroke in patients with atrial fibrillation: results from a national primary care database. J Am Coll Cardiol. 2015;66(17):1851-9.
3. Brown MP, Grundy WN, Lin D, Cristianini N, Sugnet CW, Furey TS, et al. Knowledge-based analysis of microarray gene expression data by using support vector machines. Proc Natl Acad Sci U S A. 2000;97(1):262-7.
4. Berikol GB, Yildiz O, Özcan IT. Diagnosis of acute coronary syndrome with a support vector machine. J Med Syst. 2016;40(4):84.
5. Balasubramanian V, Gouripeddi Chanathan S, Vermillion J, Bhaskaran A, Siegel R. Support vector machine based conformal predictors for risk of complications following a coronary drug eluting stent procedure. 2009 36th Annual Computers in Cardiology Conference (CinC), Park City, UT, USA; 2009. p.5-8.
6. Motwani M, Dey D, Berman DS, Germano G, Achenbach S, Al-Mallah MH, et al. Machine learning for prediction of all-cause mortality in patients with suspected coronary artery disease: a 5-year multicentre prospective registry analysis. Eur Heart J. 2017;38(7):500-7.
7. Ishwaran H, Kogalur UB, Blackstone EH, Lauer MS. Random survival forests. Ann Appl Stat. 2008;2(3):841-60.
8. Wang Y, Simon MA, Bonde P, Harris BU, Teuteberg JJ, Kormos RL, et al. Decision tree for adjuvant right ventricular support in patients receiving a left ventricular assist device. J Heart Lung Transplant. 2012;31(2):140-9.
9. Miranda E, Irwansyah E, Amelga AY, Maribondang MM, Salim M. Detection of cardiovascular disease risk's level for adults using Naive Bayes classifier. Healthc Inform Res. 2016;22(3):196-205.
10. Pal D, Mandana KM, Pal S, Sarkar D, Chakraborty C. Fuzzy expert system approach for coronary artery disease screening using clinical parameters. Knowl-Based Syst. 2012;36:162-74.
11. Saini I, Singh D, Khosla A. QRS detection using K-nearest neighbor algorithm (KNN) and evaluation on standard ECG databases. J Adv Res. 2013;4(4):331-44.
12. Karpathy A, Fei-Fei L. Deep visual-semantic alignments for generating image descriptions. IEEE Trans Pattern Anal Mach Intell. 2017;39(4):664-76.
13. Choi E, Schuetz A, Stewart WF, Sun J. Using recurrent neural network models for early detection of heart failure onset. J Am Med Inform Assoc. 2017;24(2):361-70.
14. Kannathal N, Acharya UR, Lim CM, Sadasivan PK, Krishnan S. Classification of cardiac patient states using artificial neural networks. Exp Clin Cardiol. 2003;8(4):206-11.
15. Sengupta PP, Huang YM, Bansal M, Ashrafi A, Fisher M, Shameer K, et al. Cognitive machine-learning algorithm for cardiac imaging: a pilot study for differentiating constrictive pericarditis from restrictive cardiomyopathy. Circ Cardiovasc Imaging 2016;9(6):e004330.

ORIGINAL ARTICLE

The relationship between tumor budding and survival in colorectal carcinomas

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SUMMARY

OBJECTIVE: *Tumor budding is a parameter that is increasingly understood in colorectal carcinomas. We aimed to investigate the relationship between tumor budding, prognostic factors, and survival.*

METHODS: *A total of 185 patients who had undergone colorectal surgery were observed. Tumor budding, the tumor budding score, and the relationship between these and prognostic factors, and survival investigated.*

RESULTS: *Tumor budding was found in 91 (49.2%) cases. The relationship between the tumor budding score and histological grade, lymphovascular invasion, perineural invasion, pathological lymph node stage, and mortality rates were significant.*

CONCLUSION: *In our study, the relationship between tumor budding and survival is very strong. Considering these findings and the literature, the prognostic significance of tumor budding becomes clear and should be stated in pathology reports.*

KEYWORDS: *Colorectal Neoplasms. Survival. Neoplasm Staging.*

INTRODUCTION

It is known that the pathological stage detected at the time of diagnosis in colorectal carcinoma (CRC) is the most important factor in determining the behavior and clinical course of tumors[1]. Recent studies have shown that patients at the same stage can show different prognosis, and, thus, new prognostic factors are being investigated, such as tumor budding. Tumor budding is thought to be a histological reflection of epithelial-mesenchymal transition (EMT). Cancer cells lose their epithelial properties such as polarity and adhesion during EMT; with the mesenchymal feature, they gain migratory capacity and become more resistant to apoptotic signals. The tumor cells that acquire these properties begin to separate, individually or in small groups, from the main mass[2]. Tumor budding was first described by Imai in 1954 as a morphological feature on the invasive front of the tumor, called tumor sprouting[3]. In 2002, Ueno et al. described tumor budding as a tumor cell or tumor-cell clusters of up to five cells, isolated from the main tumor on the invasive

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front. In the International Tumor Budding Consensus Conference (ITBCC), which was held on April 2016 with participants from 11 different countries, tumor budding was defined as a single tumor cell or a cell cluster of up to 4 tumor cells[4].

Numerous studies have demonstrated that tumor budding is an independent prognostic factor associated with lymph node metastasis, local recurrence, and survival. The European Society for Medical Oncology (ESMO) and ITBCC guidelines included tumor budding as a criterion for identifying high-risk patient groups[4-6].

In the present study, we aimed to investigate the presence of tumor budding and the relationship between tumor budding and prognostic factors and survival in patients with colorectal carcinoma.

METHODS

A total of 240 patients who had undergone colorectal surgery at the Haseki Training and Research Hospital in Istanbul, Turkey, between 2008-2010 were observed. Fifty-five patients were excluded from the study. Among those, 20 patients received neo-adjuvant therapy, the slides of 20 patients could not be found, 14 patients died within a month, and one patient could not be reached for survival information. Colon and rectum resection materials of 185 patients were evaluated retrospectively. All histomorphologic data was reviewed based on the corresponding hematoxylin and eosin (H&E) stained slides, whereas clinical data, tumor localization, tumor size, and surgical margins were obtained from corresponding reports. The survival information of the patients was observed from the records of the hospital information-record system and also from the patients by contacting them.

The H & E stained preparations were re-evaluated for the presence of tumor budding, tumor budding score, tumor type, grade, invasion depth, lymphovascular invasion, perineural invasion, regional lymph node involvement, pT, pN stages. Tumors were grouped as ascending colon, transverse colon, descending colon, sigmoid colon, and rectum according to their location.

We evaluated the histological subtypes following the World Health Organization (WHO) classification. Tumor invasion depth and lymph node evaluation were performed according to the 8th edition of the American Cancer Committee (AJCC) tumor-nod-metastasis (TNM) classification. The presence and score of tumor budding were assessed based on the College of American Pathologists' (CAP) colorectal carcinoma reporting protocol. Accordingly, isolated tumor cells separated from the main tumor mass on the invasive front of the tumor or tumor cell clusters of up to 5 cells were classified as tumor budding[7].

Tumor buds were counted on the invasive margin at an area of 0.785 mm^2. First, the cases were grouped as 'positive tumor budding' or 'negative tumor budding'. Then, positive tumor budding cases were scored according to the number of tumor buds. Cases with 0-4 tumor buds were scored as low grade, cases with 5-9 as moderate grade, and cases with ≥10 as high grade[4,7]. Routine immunohistochemical examination was not performed according to CAP protocol and ITBCC recommendations. Again, according to the CAP protocol and the recommendations of the ITBCC, an immunohistochemical examination was performed in 30 cases because of the inflammatory reaction surrounding the tumor, which was masking the tumor buds and the tumoral gland destruction by inflammatory cells, which simulates the tumor buds. For these cases, paraffin-embedded tissue blocks were cut at 2.5 µm thickness and immunostained for pan-cytokeratin (panCK) , a marker of epithelial cells that served to highlight areas of tumor budding.

SPSS 15.0 for Windows was used for statistical analysis. The ratios were compared by chi-square analysis, and the relationships between ratios were analyzed with Linear-by-Linear Association. The relationships between numerical and ordinal variables were analyzed by Spearman Correlation Analysis. As the numerical variables did not meet the normal distribution condition, the two groups were compared with the Mann Whitney U test. Statistical significance was accepted as $p < 0.05$.

RESULTS

A total of 185 cases were included in the study. Of these, 107 were male, and 78 were female. The mean age of the patients was 60.5 years. Tumors were localized at the ascending colon in 59 cases (31.9%), transverse colon in 11 cases (5.9%), descending colon in 27 cases (14.6%), sigmoid colon in 47 cases (25.4%), and at the rectum in 41 cases (22.2 %). The tumor diameter was 6.1 cm. The histological subgroup was classic adenocarcinoma in 170 (91.9%) cases and mucinous adenocarcinoma in 15 (8.1%) cases. The histologic grade was low in 30 (16.2%) cases, moderate in 124

(67%, and high in 31 (16.8%). Lymphovascular invasion was detected in 110 (59.5%) cases and perineural invasion in 60 (32.6%) cases. A total of 2 cases (1.1%) were evaluated as pT1, 22 cases (11.9%) as pT2, 125 (67.6%) as pT3, and 36 (19.5%) as pT4. The number of lymph nodes was between 0-44, and the average number was 14. The lymph node involvement stage was N0 in 99 (53.5%) cases, N1a in 23 (12.4%) cases, N1b in 29 (15%), N2a in 19 (10.3%), and N2b in 15 (8.1%) cases. The mean follow-up period was 68.7 months and ranged from 2 to 108 months. A total of 104 cases (56.2%) were alive, and 81 (43.8%) died.

Tumor budding was found in 91 (49.2%) cases; 49 cases (26.5%) with low grade, 17 (9.2%) with moderate, and 25 (13.5%) with high-grade tumor budding.

No significant difference was observed between tumor budding and sex, age, tumor localization, tumor size, histological type of tumor, histological grade of the tumor, or pathologic T stage ($p > 0.05$). Tumor budding was detected in 63 (69.2%) of 110 cases with lymphovascular invasion and 39 (42.9%) of 60 cases with perineural invasion. Both were significant (p=0.008 p=0.003), (Table 1).

There were significant differences between the cases with and without tumor budding in terms of pathologic lymph node stages ($p = 0.026$). İn addition, the rate of patients with tumor budding was lower than those without metastatic lymph nodes ($p = 0.0049$).

Also, the relationship between tumor budding and cumulative survival was significant; 48 (52.7%) of the 91 cases with tumor budding died, and 43 (47.3%) were still alive. The mortality rate of patients with tumor budding was significant ($p = 0.016$), as shown in Table 1.

The relationship between the tumor budding score and histological grade, lymphovascular invasion, perineural invasion, pathological lymph node stage and mortality rates were significant ($p = 0.007$, $p < 0.001$, $p < 0.001$, $p = 0.002$, $p = 0.001$). As the tumor budding scores increased, lymphovascular invasion, perineural invasion, pN, and mortality rates increased too. As the tumor budding score increased, the rates of those with histological grade 1 decreased (Table 2).

The tumor budding score was found to be positively correlated with the number of metastatic lymph nodes, amd negatively correlated with the follow-up times ($p = 0.011$ $p = 0.001$)

The cumulative survival rate of patients with tumor budding was significantly lower than of those without tumor budding ($p = 0.023$) (Table 3).

There was a significant difference in the survival rates of tumor budding scores ($p < 0.001$). The survival rates of patients with high tumor budding were significantly lower than those without tumor budding, or with low and moderate tumor budding scores. ($p < 0.001$ $p < 0.001$ $p = 0.021$) (Table 3).

TABLE 1. DISTRIBUTION OF TUMOR BUDDING BY LYMPHOVASCULAR AND PERINEURAL INVASION AND BY PN AND SURVIVAL INFORMATION

		Tumor budding				
		negative		positive		p
		n	%	n	%	
lymphovascular invasion		47	50		.0 63 69.2	0.008
perineural invasion		21	22		.6 39 42.9	0.003
pN 0		61	64.9	38	41.8	0.026
1a		7	7.4	16	17.6	
1b		12	12.8	17	18.7	
2a		7	7.4	12	13.2	
2b		7	7.4	8	8.8	
Lymph nodes Ave.±SD (Median)		14.2±9.2 (13)		13.8±8.2 (13)		1.000
Metastatic lymph nodes Ave.±SD (Median)		1.5±3.1 (0)		2.2±2.9 (1)		0.004
Follow up time Ave.±SD (Median)		72.6±32,8 (84)		64.5±36.1 (84)		0.189
Survival	alive	61	64.9	43	47.3	0.016
	death	33	35.1	48	52.7	

DISCUSSION

The number of colorectal carcinoma cases ranks 3rd worldwide. Every year, 1.4 million people are diagnosed with CRC, and more than 600 thousand people lose their lives due to the disease[8,9]. After 50 years of age, it increases significantly, reaching the highest incidence in the second half of the eighth decade[10].

The most important independent prognostic factor in CRCs is the stage of the tumor[1]. However, the fact that patients at the same pathological stage in the postoperative period present differences in terms of local recurrence and invasion suggests that pathological staging (TNM) is insufficient in these patients[11]. The situation required the investigation of biological, molecular, and morphological factors that may be related to the aggressive behavior of the tumor in the cancer tissue. Recent studies have focused on tumor budding, which is thought to be the first step of the metastatic process, among these morphological factors[12-14].

In the literature, there is no significant relationship between tumor budding, age, sex and localization, diameter, or histologic subtype of tumor; we did not find any significant relationship either[5,12,15-21].

The relationship between tumor budding and the degree of histological differentiation of the tumor was not significant. However, the relationship between the tumor budding score and the degree of histological differentiation revealed a significant decrease in the rate of good differentiation. The relationship of tumor budding with pathological T stages has been investigated in many studies, and it was found that tumor budding is associated with advanced stages[2,15-18,21-23]. Koelzer et al.[18] showed no significant relationship with pT. In our study, we found more tumor budding in pT3 stage, but this was not significant.

Few studies have demonstrated a significant correlation between tumor budding and perineural invasion[18,23,24]. In our study, a significant correlation was found between the presence of tumor budding and the score of tumor budding and perineural invasion.

A significant relationship between tumor budding and lymphovascular invasion has been shown in most studies[2,15-18,21,23,24]. In our study, the relationship between tumor budding and lymphovascular invasion

TABLE 2. THE RELATIONSHIP BETWEEN THE TUMOR BUDDING SCORE AND CLINICOPATHOLOGICAL PARAMETERS

		Tumor budding								
		negative		low		moderate		high		
		n	%	n	%	n	%	n	%	p
Histologic grade	1	20	21.3	8	16.3	1	5.9	1	4.0	0.007
	2	59	62.8	36	73.5	14	82.4	15	60.0	
	3	15	16.0	5	10.2	2	11.8	9	36.0	
lymphovascular invasion		47	50	27	55.1	13	76.5	23	92	<0.001
perineural invasion		21	22.6	17	34.7	5	29.4	17	68	<0.001
pN	0	61	64.9	27	55.1	4	23.5	7	28.0	0.002
	1a	7	7.4	8	16.3	4	23.5	4	16.0	
	1b	12	12.8	6	12.2	3	17.6	8	32.0	
	2a	7	7.4	5	10.2	3	17.6	4	16.0	
	2b	7	7.4	3	6.1	3	17.6	2	8.0	
Survival	alive	61	64.9	28	57.1	9	52.9	6	240	0.001
	death	33	35.1	21	42.9	8	47.1	19	76.0	

TABLE 3. THE RELATIONSHIP BETWEEN TUMOR BUDDING AND SURVIVAL

		Cumulative survival(%)				
		12 month	36 month	60month	100 month	Log Rank p
Tumor budding	negative	95.7	89.4	70.2	64.0	0.023
	positive	89.0	69.2	56.0	47.3	
Tumor budding score	negative	93.7	76.6	70.2	64.0	<0.001
	low	93.9	79.6	67.3	58.3	
	moderate	100.0	76.5	58.8	50.4	
	high	72.0	44.0	32.0	24.0	

was significant. In addition, as the degree of tumor budding increases, the rate of cases with lymphovascular invasion increases too, so there is a significant relationship between the tumor budding score and lymphovascular invasion.

Numerous studies have examined the relationship of tumor budding with lymph node metastasis. In 2012, Kye et al.[25] compared the relationship between regional lymph node metastasis and all other prognostic factors in 55 patients with stage pT1; among all parameters, only tumor budding was found to be an independent prognostic factor for lymph node metastasis. In our study, the relationship of tumor budding with metastatic lymph node number and lymph node stage was significant. In addition, the relationship between the tumor budding score and pN stage is significant; as the degree of tumor budding increases, the pN is also increased.

As the prognostic importance of tumor budding was understood, the number of studies investigating the relationship with survival also increased. All these studies have shown that the presence of tumor budding is associated with significant poor clinical outcomes and shorter survival time.

In the study by Ohtsuki et al.[23], the disease-free survival rate was 40.9% for cases with tumor budding and 75.1% in cases without tumor budding. Rogers et al.[26] reported that tumor budding was an independent prognostic factor related to cancer-related death in univariate and multivariate analyses. When we look at the relationship between tumor budding and cumulative survival, of the 91 cases with tumor budding, 48 (52.7%) died, and 43 (47.3%) were still alive. The mortality rate of patients with tumor budding was significant. There was a significant correlation between the tumor budding degrees and follow-up periods in patients with tumor budding. The cumulative survival rate of patients with tumor budding was significantly lower than those without tumor budding. In addition, the relationship between the score of tumor budding and cumulative survival is significant. The cumulative survival rate of patients with high tumor budding rates is significantly lower than other scores and no tumor budding.

CONCLUSION

In our study, the relationship between tumor budding and survival is very strong. Considering these findings and the literature, the prognostic significance of tumor budding becomes clear and should be stated in pathology reports.

RESUMO

OBJETIVO: *Brotamento de tumor é um parâmetro que é cada vez mais conhecido em carcinomas colorretais. Nosso objetivo foi investigar a relação entre brotamento tumoral e fatores prognósticos e sobrevida.*

MÉTODOS: *Um total de 240 pacientes observados, submetidos à cirurgia colorretal. Brotamento de tumor, escore de brotamento tumoral e a relação entre estes e fatores prognósticos, sobrevida investigada.*

RESULTADOS: *Brotamento de tumores foi encontrado em 91 (49,2%) casos. A relação entre o escore de brotamento tumoral e o grau histológico, invasão linfovascular, invasão perineural, estadiamento linfonodal patológico e taxas de mortalidade foram significativas.*

CONCLUSÃO: *Em nosso estudo, a relação entre brotamento tumoral e sobrevida é muito forte. Em conjunto, todos esses achados e literatura são avaliados simultaneamente, o significado prognóstico da brotação do tumor é claramente visto e deve ser indicado nos relatórios de patologia.*

PALAVRAS-CHAVE: *Neoplasias Colorretais. Sobrevida. Estadiamento de Neoplasias.*

REFERENCES

1. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017;67(2):93-9.
2. Zlobec I, Lugli A. Epithelial mesenchymal transition and tumor budding in aggressive colorectal cancer: tumor budding as oncotarget. Oncotarget. 2010;1(7):651-61.
3. Okuyama T, Oya M, Yamaguchi M. Budding (sprouting) as a useful prognostic marker in colorectal mucinous carcinoma. Jpn J Clin Oncol. 2002;32(10):412-6.
4. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017;30(9):1299-311.
5. Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer: ready for diagnostic practice? Hum Pathol. 2016;47(1):4-19.
6. Schmoll H, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012;23(10):2479-516.
7. Cho SJ, Kakar S. Tumor budding in colorectal carcinoma: translating a morphologic score into clinically meaningful results. Arch Pathol Lab Med. 2018;142(8):952-7.
8. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012. [cited 2019 May 12]. Available from: https://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012
9. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.
10. Richman SD, Southward K, Chambers P, Cross D, Barrett J, Hemmings G, et al. HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. J Pathol. 2016;238(4):562-70.
11. Park KJ, Choi HJ, Roh MS, Kwon HC, Kim C. Intensity of tumor budding and its prognostic implications in invasive colon carcinoma. Dis Colon Rectum. 2005;48(8):1597-602.
12. Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC. Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology. 2002;40(2):127-32.
13. Nakamura T, Mitomi H, Kikuchi S, Ohtani Y, Sato K. Evaluation of the usefulness of tumor budding on the prediction of metastasis to the lung and liver after curative excision of colorectal cancer. Hepatogastroenterology. 2005;52(65):1432-5.
14. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, et al. Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology. 2004;127(2):385-94.
15. Graham RP, Vierkant RA, Tillmans LS, Wang AH, Laird PW, Weisenberger DJ, et al. Tumor budding in colorectal carcinoma: confirmation of prognostic significance and histologic cutoff in a population-based cohort. Am J Surg Pathol. 2015;39(10):1340-6.
16. Kazama S, Watanabe T, Ajioka Y, Kanazawa T, Nagawa H. Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5. 2. Br J Cancer. 2006;94(2):293-8.
17. Satoh K, Nimura S, Aoki M, Hamasaki M, Koga K, Iwasaki H, et al. Tumor budding in colorectal carcinoma assessed by cytokeratin immunostaining and budding areas: possible involvement of c-Met. Cancer Sci. 2014;105(11):1487-95.
18. Koelzer VH, Assarzadegan N, Dawson H, Mitrovic B, Grin A, Messenger DE, et al. Cytokeratin-based assessment of tumour budding in colorectal cancer: analysis in stage II patients and prospective diagnostic experience. J Pathol Clin Res. 2017;3(3):171-8.
19. Horcic M, Koelzer VH, Karamitopoulou E, Terracciano L, Puppa G, Zlobec I, et al. Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol. 2013;44(5):697-705.
20. Nakamura T, Mitomi H, Kanazawa H, Ohkura Y, Watanabe M. Tumor budding as an index to identify high-risk patients with stage II colon cancer. Dis Colon Rectum. 2008;51(5):568-72.
21. Karamitopoulou E, Zlobec I, Kölzer V, Kondi-Pafiti A, Patsouris ES, Gennatas K, et al. Proposal for a 10-high-power-fields scoring method for the assessment of tumor budding in colorectal cancer. Mod Pathol. 2013;26(2):295-301.
22. Zlobec I, Hädrich M, Dawson H, Koelzer V, Borner M, Mallaev M, et al. Intratumoural budding (ITB) in preoperative biopsies predicts the presence of lymph node and distant metastases in colon and rectal cancer patients. Br J Cancer. 2014;110(4):1008-13.
23. Ohtsuki K, Koyama F, Tamura T, Enomoto Y, Fujii H, Mukogawa T, et al. Prognostic value of immunohistochemical analysis of tumor budding in colorectal carcinoma. Anticancer Res. 2008;28(3B):1831-6.
24. Wang LM, Kevans D, Mulcahy H, O'Sullivan J, Fennelly D, Hyland J, et al. Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. Am J Surg Pathol. 2009;33(1):134-41.
25. Kye BH, Jung JH, Kim HJ, Kang SG, Cho HM, Kim JG. Tumor budding as a risk factor of lymph node metastasis in submucosal invasive T1 colorectal carcinoma: a retrospective study. BMC Surg. 2012;12:16.
26. Rogers AC, Gibbons D, Hanly AM, Hyland JM, O'Connell PR, Winter DC, et al. Prognostic significance of tumor budding in rectal cancer biopsies before neoadjuvant therapy. Mod Pathol. 2014;27(1):156-62.

ORIGINAL ARTICLE

Is neutrophil to lymphocyte ratio a predictive factor for recurrence of urethral stricture?

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SUMMARY

INTRODUCTION: *Due to spongiofibrosis and inflammatory processes underlying the pathogenesis of urethral stricture, it is possible that the neutrophil-lymphocyte ratio (NLR) may give essential information about the course of the disease and recurrence possibilities. Our study aims to evaluate the correlation between NLR and recurrence rates.*

METHODS: *A total of 512 patients who underwent direct visual internal urethrotomy (DVIU) due to urethral stricture in our clinic between February 2010 and January 2018 were evaluated retrospectively.*

RESULTS: *The median follow up for non-recurrent and recurrent groups after DVIU was 30 and 36 months, respectively. During the follow-up, 280 (54.7%) of the patients had recurrences, and 232 (45,3%) had no recurrences. The mean time for recurrence after DVIU was 6,5±1,4 months, with a range of 1-36 months. The mean NLR in the non-recurrence group was 2,02±0,87, with a median of 1.9, and 3,66±2,30, with a median of 3 in the recurrence group. A highly significant statistical difference was observed between two groups in terms of neutrophil count and NLR (p: 0.000 – both). The area under curve value for NLR was 0.767, with a standard error of 0.021 (95% CI 0.727-0.808). The cut-off value of NLR was determined as 2.25, with a 70% sensitivity and 67,7% specificity.*

CONCLUSION: *By using NLR, the inflammatory features of the urethral tissue can be predicted, and possible recurrences after surgery can be estimated. Consequently, open urethroplasty techniques can be used in cases with a significant NLR value instead of the recurrent endoscopic procedure.*

KEYWORDS: *Inflammation. Urethra/surgery. Recurrence. Urethral stricture. Urologic Surgical Procedures, Male.*

INTRODUCTION

Urethral stricture is a relatively common and debilitant disease, with an incidence of 0.6%, which occurs due to several different etiologic reasons in different age groups.[1] Etiology mostly consists of iatrogenic reasons (catheterization/endoscopic procedures), as well as trauma, infections, prostatectomy, and other post-prostate cancer treatments, lichen sclerosis, and idiopathic reasons.[2] Its incidence is higher in older and African-American men.[1] The main pathophysiological factor for urethral stricture is spongiofibrosis, causing scar tissues in the corpus spongiosum and narrowing the urethral lumen as a

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result. The process leading to this fibrosis is primarily subepithelial inflammation and hemorrhage, and later stages are characterized as sclerosis and fibrosis.[3] The latest molecular studies show that there is a disproportion between collagen type 1 over collagen type 3. It has also been found that this spongiofibrotic process may be induced by inflammatory mediators such as neuronal nitric oxide synthase 1 and transforming growth factor beta (TGF-β) in connective tissue.[4,5]

White blood cells differentiate as neutrophils and lymphocytes as a response to inflammation. Elevated neutrophil to lymphocyte ratio (NLR) is associated with chronic inflammation and found to be a marker to estimate the progression and prognosis of many diseases and types of cancer.[6-8] Since the endoscopic procedures are a factor of urethral stricture, which disfigures the smoothness and continuity of the urethral mucosa and the microstructure of the corpus spongiosum, recurrence is frequent. Recurrence rates vary from 23-92% in endoscopic procedures and 5-14% in open reconstructive surgery techniques.[9,10] Direct visual internal urethrotomy (DVIU) is the most common type of surgery in cases of strictures mostly in the bulbar urethra and short stricture length.[9] Its lower complexity and effort-time efficiency make this technique an eligible choice.[11]

Due to the spongiofibrosis and inflammatory processes underlying the pathogenesis of urethral stricture, NLR may give essential information about the course of the disease and recurrence possibilities. Our study aims to evaluate the correlation between NLR and recurrence rates.

METHODS

After obtaining approval by the institutional review board, 512 patients who underwent DVIU due to urethral stricture in our clinic between February 2010 and January 2018 were evaluated retrospectively. Patients' age at the time of the surgery, location, and length of stenosis in the operation reports, follow-up period, and previous DVIU history of the patients who were referred from outer centers were recorded. In addition, neutrophil, lymphocyte, neutrophil/lymphocyte ratio, hematocrit, and albumin levels were recorded from complete blood count and biochemistry analyses done for preoperative anesthesia assessment. After a descriptive analysis of the patients, comparative statistics were performed in 2 groups, one with no recurrence after the DVIU procedure, and the other with one or more recurrence. Patients with malignancies, uncontrolled diabetes mellitus, hematologic, liver, or kidney dysfunction, who received blood transfusions, and with a previous open urethral surgery were excluded. Urine culture tests were performed on each patient preoperatively, and the surgeries of patients with active infections were postponed until the treatment was completed. Blood counts of all patients were made on a stationary device in the hospital's central laboratory, which is regularly checked.

In our clinic, DVIU is applied as a standard to patients who are surgery-naïve and have short segment bulbar urethral stricture (<1.5 cm, mostly <1 cm). After admitting an appropriate prophylactic single dose of antibiotics, all operations were performed in a lithotomy position. The standard procedure of DVIU in our clinic is performed with a single incision using a cold-knife at 12 o'clock position. A 16-18F silicone catheter is applied after the surgery and is removed routinely on the 2nd day postoperatively. Open urethroplasty is the choice of treatment in long segment strictures and recurrence after DVIU. Our clinic is a reference center for open urethroplasty, and patients with recurrent strictures after DVIU are referred from many other clinics. Most of the patients included in our study with one or more recurrence after DVIU consists of these kinds of patients. In our clinic, self-catheterization or bougie dilatation procedure is not applied to avoid or delay recurrences. Patients underwent clinical evaluation and uroflowmetry every three months for one year after DVIU and, later, every six months for two years. The time for recurrence was defined as the time from DVIU to the first clinical sign of recurrence (symptoms and uroflowmetric evaluation) or the date of subsequent repeat DVIU or urethroplasty surgery.

STATISTICAL ANALYSIS

Statistical analysis was performed using the IBM SPSS Statistics 25 software. The study data was evaluated by the Shapiro-Wilks test in terms of distribution, and the parameters were assessed as not normally distributed. Descriptive statistical methods (mean, standard deviation, frequency), as well as the Mann-Whitney U test, were used for the comparison of the two groups when evaluating the study data. $P < 0.05$ was assessed as significance.

RESULTS

A total of 512 patients were included in the study. The mean age of the patients with and without recurrence after DVIU was 52,9±11,6 and 50,6±10,8, respectively. There was no significant difference between the mean ages of both groups. The mean follow-up for the non-recurrent group after DVIU was 31,7±6,4 months, with a range of 6-63 months, and the median follow up was 30 months. The mean follow-up for the recurrent group after DVIU was 35,2±9,1 months with a range of 6-72 months, and the median follow up was 36 months. During the follow-up, after the first DVIU procedure, 280 (54.7%) of the patients had recurrences, and 232 (45,3%) had no recurrences (Table 1). The mean time to recurrence after DVIU was 6,5±1,4 months with a range of 1-36 months, and the median time to recurrence was seven months. The total number of recurrences seen in patients varies from 1 to 7, with an average of 1.83±1.34 and a median of 1. Neutrophil, lymphocyte, and hematocrit levels were present in all patients since they were routinely performed during anesthesia assessment, while preoperative albumin values were present in 249 of 512 patients. A total of 144 of the 249 patients were in the non-recurrence group, and 105 were in the recurrence group.

TABLE 1. EVALUATION OF BASIC PARAMETERS, HEMATOLOGIC PARAMETERS AND NLR ACCORDING TO THE PRESENCE OF RECURRENCE

	Recurrence	
	No (n:232)	Yes (n:280)
	Mean±SD (Range)	Mean±SD (Range)p
Patient age (year)	52.9±11.6 (23-78)	50.6±10.8 (21-75)
Follow up (months)	31.7±6.4 (6-63)	35.2±9.1 (6-72)
Time to recurrence (months)		6.5±1.4 (1-36)

Neutrophil (mean)	4		.43±1.61 (4.41) 5.79±2.34 (5.4) 0.000*
Lymphocyte (mean)	2		.38±1.08 (2.24) 1.87±0.80 (1.79) 0.000*
NLR (mean)	2		.02±0.87 (1.9) 3.66±2.30 (3.04) 0.000*
Hematocrit (mean)	40		.31±5.27 (41.4) 40.24±5.69 (40.9) 0.950
Albumin (mean)	4.2±3.32 (4)	3 .91±0.41 (3.9)	0.531

Mann Whitney U Test. NLR: neutrophil to lymphocyte ratio * p<0.005

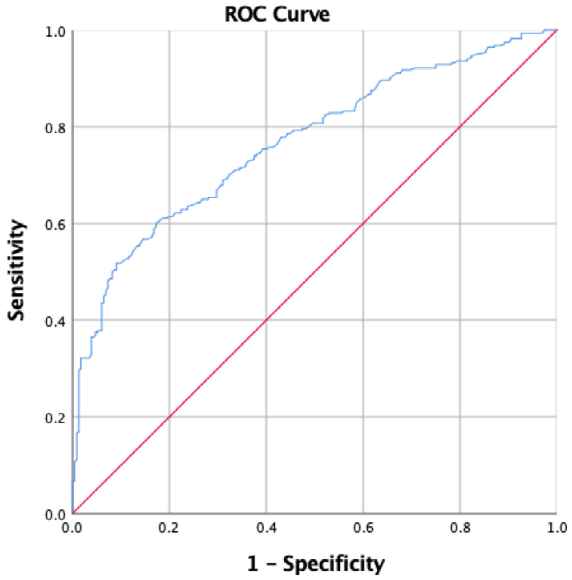
In the non-recurrence group, mean neutrophil levels were 4,43±1,61, with a median of 4.4. The mean neutrophil levels in the recurrence group were 5,79±2,34, with a median of 5.4. The mean lymphocyte levels in the non-recurrence and recurrence groups were 2,38±1,08, with a median of 2.2 and 1,87±0,8 with a median of 1.8, respectively. The mean NLR in the non-recurrence group was 2,02±0,87 with a median of 1.9, and 3,66±2,30 with a median of 3 in the recurrence group. The mean hematocrit levels were 40,31±5,27 (median 41.4) and 40,24±5,69 (median 40.9) respectively. Finally, mean albumin levels were 4,2±3,32 (median 4) in the non-recurrence group, and 3,91±0,41 (median 3.9) in the recurrence group. A highly significant statistical difference was observed between two groups in terms of neutrophil count and neutrophil-lymphocyte ratio. (p: 0.000 – both). The area under curve (AUC) value for NLR was 0.767, with a standard error of 0.021 (95% CI 0.727-0.808)(Figure 1). The cut-off value of NLR in the detection of recurrence after internal urethrotomy was determined as 2.25, with a 70 % sensitivity, 67,7 % specificity. There was no statistically significant difference between the groups in terms of lymphocyte, hematocrit, and albumin levels (p>0.05)(Table 1).

DISCUSSION

Managing urethral strictures is a challenge for surgeons and can be a burden to both the patient and the surgeon. It is still uncertain which patients need which kind of procedure. Endoscopic interventions are simple and easy to apply but have high recurrence rates. Contrarily, open techniques are promising with better long-term results but require high expertise, precise technique, and special instrumentation.[12] Predicting the recurrence rates before deciding on the technique for each patient can help surgeons offer the most beneficial procedure. While many studies on NLR have been performed in many different fields in the literature, as far as we know, our study is the first involving urethral strictures.

In recent years, it has been shown that NLR may be a marker of chronic systemic inflammation and is associated with prognosis in many cardiovascular diseases, malignancies, and chronic inflammatory diseases. These markers were previously assessed in various uro-oncologic cases and have been shown to have an effective role in both predicting postoperative

FIGURE 1. ROC CURVE OF NEUTROPHIL-LYMPHOCYTE RATIO IN THE PREDICTION OF RECURRENCE AFTER INTERNAL URETHROTOMY



surgical margin status and progression-free survival.[13,14] In a study that regarded a NLR of 2.7 as a limit, it was shown that a combination of tumor stage and NLR could be used to assess the risk of recurrence in patients with non-metastatic renal cell carcinoma.[15] Another study showed that NLR in non-clear-cell kidney tumors was an independent prognostic factor for disease-free survival after curative surgery. As such, NLR has been reported to be a significant marker for patient counseling and clinical trial design.[16]

Inflammation, especially chronic inflammation in organs with lumens, can lead to stenosis and obstruction by causing fibrosis. Based on this hypothesis, inflammation markers can be used as a predictive factor in the development of restenosis and obstruction. In a study conducted by Qian et al.17 on 261 coronary artery patients, inflammation markers were evaluated in groups with and without restenosis, and it concluded that these markers could be used as an independent predictive factor for restenosis development. In 2019, Velioglu and Yuksel[18] investigated the relationship between inflammation and peripheral arterial disease. They concluded that the higher the NLR, which is used as an indicator of inflammation, the higher the severity of the peripheral arterial disease. In this study, the severity of inflammation in tissues with a lumen is used to predict the pathology inside the lumen. This has been evaluated in internal urethrotomy patients. By using NLR, especially in frequent and multiple recurrent patients, the inflammatory features of the tissue could be predicted and possible recurrences after surgery estimated.

Recurrences after DVIU can be due to infection and inflammation after the operation. When urethral strictures are considered, fibroblasts are probably responsible for the development of the urethral stricture; however, the reason for the urethral stricture is related to the urinary extravasation into the subepithelial space causing increased inflammation and subsequent scar formation. With this knowledge, many authors have used colchicine, mitomycin-C, triamcinolone, corticosteroids, and anti-inflammatory drugs locally or systemically to reduce urethral stricture after urethral procedures.[19] In this context, the urethral stricture is a result of inflammatory changes in the epithelium of the urethra and can be treated by interfering with the inflammatory process. We used anti-inflammatory drugs (COX-2 inhibitors) for three days after the operation to reduce inflammation in our cases.

In patients with urethral stricture, anti-fibrosis agents were used to prevent a recurrence. In a very recent study conducted in 2018, patients who received 10 mg tamoxifen daily for six months after internal urethrotomy were compared with the control group, and tamoxifen was shown to significantly reduce re-fibrosis and stricture development. Again in 2016, in a study with 83 patients, Yıldırım et al.[20] showed that recurrence is significantly lower in patients who received a local urethral steroid injection compared with a control. Sinanoglu et al.[21] conducted a similar study with 84 patients and used colchicine as an anti-inflammatory agent, and the recurrence in this group was significantly lower.

It is known that white blood cells differ in systemic inflammation, such as neutrophilia and lymphopenia.[6] This inflammatory response and tissue necrosis leads to fibrosis and poor recipient vascularity, which likely has a key role in deficient wound healing, which, in turn, threatens urethroplasty success.[22] In a related study of 208 patients with a history of urethral stricture after transurethral resection of the prostate, it was shown that the NLR was relatively higher in relapsed patients but not significant.[19] Chronic inflammation in the urethral tissue, as well as inflammation of the prostate tissue, may be effective in the development of urethral stricture, especially after endoscopic procedures. Grechenkov et al.[23] found that patients who have chronic prostatic

inflammation after TUR-P have a significantly higher risk of developing urethral stricture than patients without prostatic inflammation.

Acute or chronic inflammation-fibrosis-sclerosis may be effective in the recurrence of urethral strictures. Some histological studies support this theory. Ekerhult et al.[24] examined the stenosis segment histopathologically in 45 patients with open urethroplasty and found a significantly increased risk of developing stenosis after urethroplasty in patients with sclerosis, compared to those without it. Inflammation and established sclerosis are prominent, especially in patients with recurrent urethral strictures. Recurrent endoscopic urethrotomy procedures in sclerotic tissue may cause recurrence and not provide curative results. In these cases, NLR can be used as a predictive value. Open urethroplasty techniques can be used in cases with a significant NLR value by using grafts or flaps instead of recurrent endoscopic procedures. Also, in our study, there were statistically significant differences between neutrophils and NLR in patients with and without stricture recurrence after DVIU.

Our study is not free of limitations because of its retrospective design. There are deficiencies related to the previous follow-up of patients referred to our clinic from outer centers, especially after repeated urethrotomy procedures from outer centers. The main limitations of our study are its single-center design, the relatively limited number of patients, its retrospective nature, and the exclusion of older patients due to additional comorbidities.

By using NLR, the inflammatory features of the urethral tissue can be predicted, and possible recurrences after surgery can be estimated. In this manner, open urethroplasty techniques can be used in cases with a significant NLR value instead of the recurrent endoscopic procedure.

Ethical Approval: All procedures performed in studies involving human participants were per the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The authors declare they have no conflicts of interest.

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Authors' contributions

AU, RT, and MAK conceived the study, participated in its design and coordination, drafted the manuscript, and revised it critically for important intellectual content. EO, ET, RK, and IA participated in the design of the study and made substantial contributions to the acquisition and interpretation of data. MIO participated in its design and drafted the manuscript. All authors read and approved the final manuscript.

CONCLUSION

RESUMO

INTRODUÇÃO: *Devido à espongiofibrose e processos inflamatórios subjacentes à patogênese da estenose uretral, pode-se pensar que a relação de linfócitos neutrofílicos (NLR) pode fornecer informações essenciais sobre o curso da doença e as possibilidades de recorrência. O objetivo do nosso estudo é avaliar a correlação entre NLR e taxas de recorrência.*

MÉTODOS: *Quinhentos e doze pacientes submetidos à uretrotomia interna visual direta (DVIU) devido à estenose uretral em nossa clínica entre as datas de fevereiro de 2010 e janeiro de 2018 foram avaliados retrospectivamente.*

RESULTADOS: *A mediana de acompanhamento para os grupos não recorrentes e recorrentes após a DVIU foi de 30 e 36 meses, respectivamente. Durante o seguimento, 280 (54,7%) dos pacientes tiveram recidivas e 232 (45,3%) não tiveram recidivas. O tempo médio de recorrência após a DVIU foi de 6,5±1,4 mês, com variação de 1-36 meses. A média da RNL no grupo sem recorrência foi de 2,02±0,87 com mediana de 1,9 e 3,66±2,30 com mediana de 3 no grupo com recidiva. Uma diferença estatística altamente significativa foi observada entre dois grupos em termos de contagem de neutrófilos e NLR (p: 0,000 - ambos). A área sob o valor da curva para NLR foi de 0,767 com um erro padrão de 0,021 (IC 95% 0,727-0,808). Valor de corte de NLR determinado como 2,25 com uma sensibilidade de 70%, especificidade de 67,7%.*

CONCLUSÃO: *Ao utilizar a RNL, as características inflamatórias do tecido uretral podem ser previstas e possíveis recidivas após a cirurgia podem ser estimadas. Dessa forma, técnicas de uretroplastia aberta podem ser usadas em casos com valor significativo de NLR em vez de procedimento endoscópico recorrente.*

PALAVRAS-CHAVE: *Inflamação. Uretra/cirurgia. Recidiva. Estreitamento uretral. Procedimentos cirúrgicos urológicos masculinos.*

REFERENCES

1. Alwaal A, Blaschko SD, McAninch JW, Breyer BN. Epidemiology of urethral strictures. Transl Androl Urol. 2014;3(2):209-13.
2. Lumen N, Hoebeke P, Willemsen P, De Troyer B, Pieters R, Oosterlinck W. Etiology of urethral stricture disease in the 21st century. J Urol. 2009;182(3):983-7.
3. Hampson LA, McAninch JW, Breyer BN. Male urethral strictures and their management. Nat Rev Urol. 2014;11(1):43-50.
4. Baskin LS, Constantinescu SC, Howard PS, McAninch JW, Ewalt DH, Duckett JW, et al. Biochemical characterization and quantification of the collagenous components of urethral stricture tissue. J Urol. 1993;150(2 Pt 2):642-7.
5. Zhang P, Shi M, Wei Q, Wang K, Li X, Li H, et al. Increased expression of connective tissue grow factor in patients with urethral stricture. Tohoku J Exp Med. 2008;215(3):199-206.
6. Aktas G, Sit M, Dikbas O, Erkol H, Altinordu R, Erkus E, et al. Elevated neutrophil-to-lymphocyte ratio in the diagnosis of Hashimoto's thyroiditis. Rev Assoc Med Bras. 2017;63(12):1065-8.
7. Costa CH, Rufino R, Lapa e Silva JR. Inflammatory cells and their mediators in COPD pathogenesis. Rev Assoc Med Bras. 2009;55(3):347-54.
8. Yuksel OH, Verit A, Sahin A, Urkmez A, Uruc F. White blood cell counts and neutrophil to lymphocyte ratio in the diagnosis of testicular cancer: a simple secondary serum tumor marker. Int Braz J Urol. 2016;42(1):53-9.
9. Kluth LA, Ernst L, Vetterlein MW, Meyer CP, Reiss CP, Fisch M, et al. Direct vision internal urethrotomy for short anterior urethral strictures and beyond: success rates, predictors of treatment failure, and recurrence management. Urology. 2017;106:210-5.
10. Sukumar S, Elliott SP, Myers JB, Voelzke BB, Smith TG 3rd, Carolan AMC, et al. Multi-institutional outcomes of endoscopic management of stricture recurrence after bulbar urethroplasty. J Urol. 2018;200(4):837-42.
11. Tolkach Y, Herrmann T, Merseburger A, Burchardt M, Wolters M, Huusmann S, et al. Development of a clinical algorithm for treating urethral strictures based on a large retrospective single-center cohort. Version2. F1000Res. 2016;5:2378.
12. Ferguson GG, Bullock TL, Anderson RE, Blalock RE, Brandes SB. Minimally invasive methods for bulbar urethral strictures: a survey of members of the American Urological Association. Urology. 2011;78(3):701-6.
13. Krane LS, Richards KA, Kader AK, Davis R, Balaji KC, Hemal AK. Preoperative neutrophil/lymphocyte ratio predicts overall survival and extravesical disease in patients undergoing radical cystectomy. J Endourol. 2013;27(8):1046-50.
14. Grimes N, Hannan C, Tyson M, Thwaini A. The role of neutrophil-lymphocyte ratio as a prognostic indicator in patients undergoing nephrectomy for renal cell carcinoma. Can Urol Assoc J. 2018;12(7):E345-8.
15. Ohno Y, Nakashima J, Ohori M, Hatano T, Tachibana M. Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma. J Urol. 2010;184(3):873-8.
16. Martino M, Pantuck AJ, Hofbauer S, Waldert M, Shariat SF, Belldegrun AS, et al. Prognostic impact of preoperative neutrophil-to-lymphocyte ratio in localized nonclear cell renal cell carcinoma. J Urol. 2013;190:1999-2004.
17. Qian H, Luo Z, Xiao C, Chen J, Li D, Xu H, et al. Red cell distribution width in coronary heart disease: prediction of restenosis and its relationship with inflammatory markers and lipids. Postgrad Med J. 2018;94(1115):489-94.
18. Velioglu Y, Yuksel A. Complete blood count parameters in peripheral arterial disease. Aging Male. 2019;22(3):187-91.
19. Gül M, Altıntaş E, Kaynar M, Buğday MS, Göktaş S. The predictive value of platelet to lymphocyte and neutrophil to lymphocyte ratio in determining urethral stricture after transurethral resection of prostate. Turk J Urol. 2017;43(3):325-9.
20. Yıldırım ME, Kaynar M, Ozyuvali E, Badem H, Cakmak M, Kosem B, et al. The effectiveness of local steroid injection after internal urethrotomy to avoid recurrence. Arch Ital Urol Androl. 2016;87(4):295-8.
21. Sinanoglu O, Kurtulus FO, Akgün FS. Long-term effect of colchicine treatment in preventing urethral stricture recurrence after internal urethrotomy. Urol J. 2018;15(4):204-8.
22. Chapman D, Kinnaird A, Rourke K. Independent predictors of stricture recurrence following urethroplasty for isolated bulbar urethral strictures. J Urol. 2017;198(5):1107-12.
23. Grechenkov A, Sukhanov R, Bezrukov E, Butnaru D, Barbagli G, Vasyutin I, et al. Risk factors for urethral stricture and/or bladder neck contracture after monopolar transurethral resection of the prostate for benign prostatic hyperplasia. Urologia. 2018;85(4):150-7.
24. Ekerhult TO, Lindqvist K, Grenabo L, Kåbjörn Gustafsson C, Peeker R. Sclerosis as a predictive factor for failure after bulbar urethroplasty: a prospective single-centre study. Scand J Urol. 2018;52(4):302-8.

ORIGINAL ARTICLE

Quality of sleep and use of computers and cell-phones among university students

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SUMMARY

OBJECTIVE: *Evaluate the quality of sleep and its association with the use of computers and cell-phones among medicine and dentistry students.*

METHODS: *Cross-sectional and comparative study, which evaluated 425 students through a socioeconomic questionnaire, the Pittsburgh Sleep Quality Index(PSQI), and a questionnaire on their use of computers and cell phones.*

RESULTS: *Poor sleep quality was observed in 61.4% of medical students and in 60.1% of dentistry students. Medical students with poor sleep quality had a higher mean time of computer use at night when compared to those with good sleep quality (p=0.04), as well as for computer (p<0.001) and cell phone use (p<0.01) immediately before bedtime. Dentistry students with poor sleep quality had a higher average time of computer use before bedtime than those with good sleep quality (p=0.03).*

CONCLUSION: *Students should receive guidance on prevention strategies and quality of sleep care.*

KEYWORDS: *Sleep. Students, Medical. Students, Dental. Technology.*

INTRODUCTION

The quality of sleep of health students[1-4] has been studied, and some evidence suggests that their poor sleep quality can be associated with the use of computers at night[5] and the excessive use of cell phones[6].

University students are recognized as one of the groups with greater sleep deprivation and one of the most technologically-oriented[7]. The use immediately before bedtime of portable electronic devices that emit light, depending on its intensity, variation, and duration projected on the retina, can lead to the inhibition of the melatonin secretion. These biological effects can perpetuate sleep deficiency and disrupt the circadian rhythm, with consequences on performance, health, and safety[8].

This is a subject of great importance since the use of technology is present in many aspects of the life of young adults, and its impact is still uncertain. In addition, data from Brazilian literature on the quality of sleep and its association with the use of computers and cell phones by health university students are still limited. This study aimed to evaluate the quality of sleep and its association with the use of computers and cell phones in medical and dentistry students.

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METHODS

This is a cross-sectional and comparative study, conducted at a university in southern Brasil. A total of 243 medical students and 294 dentistry students were regularly enrolled. A total of 21 medical students and 52 dentistry students did not participate because they were not in the classroom or were not available due to internship activities at the time of collection, as well as in another attempt to include them. We excluded 18 students under the age of 18 years and data of 21 questionnaires that were incomplete. Thus, we analyzed 425 students, 207 of medicine (85.2%) and 218 of dentistry (74.2%).

The collection was carried out between March and April 2017, period in which no tests were being carried out. We collected socioeconomic and quality of sleep data using the Pittsburgh Sleep Quality Index (PSQI), translated and validated into Portuguese. Based on the global score, between 0 and 21, students quality of sleep with scores >5 were classified as poor and ≤5 as good[9]. Since we found no instrument in the literature validated on the use of mobile phones and computers, we drew up questions about the use of cell phones and computers, referring to the previous 30 days, on average time of use of computers and cell phones in 24 hours, during the night (between 18h and 6h), and immediately before bedtime.

The association between categorical variables was performed by Pearson's chi-square test and Fisher's exact test. Comparisons regarding the quality of sleep were adjusted for the variables age, gender, and income, when necessary, using logistic regression (enter method). Continuous variables were compared using the Mann-Whitney test, with their normality checked by histograms and the Shapiro-Wilk test.

We used the Statistical Package for Social Sciences (SPSS15.0) with a significance level of 5%. The study was approved by the Research Ethics Committee, decision 1,846,977 and CAAE 62388416.3.0000.5215

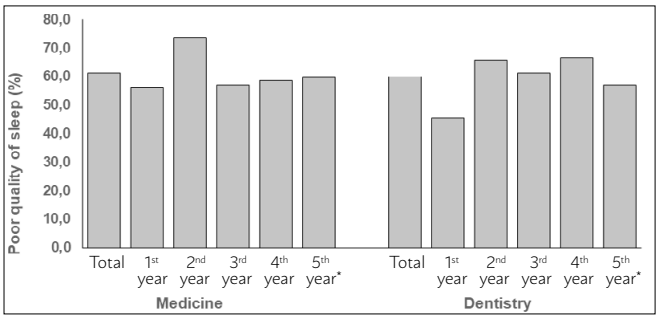
RESULTS

Most students of medicine were aged ≥21 years (78.3%), male (53.6%), and single (95.2%). Dentistry students were aged <21 years (54.6%), female (73.9%), and single (98.2%). Most had a monthly per capita household income ≤R$ 2,000.00 (57.0% for medicine and 71.5% for dentistry). In comparison, dentistry students had a greater proportion of age <21 years (p<0.001), females (p<0.001), and lower-income (p<0.01).

Poor sleep quality (PSQI-BR) was found in 61.4% of the total number of students of medicine and in 60.1% of dentistry students (Figure 1). Both medical and dentistry students reported, respectively, an average duration of sleep per night of 6.3(+1.1) and 6.7(+1.1) hours, taking 23.2(+24.9) and 23.8(+19.6) minutes to fall asleep, and 9.7% and 8.7% used medication to help them sleep once or more times per week.

A sleep efficiency greater than 85% was observed in 78.3% of medical students and 77.1% of dentistry students (p=0.47). Moderate and severe daytime dysfunction was 49.3% for medical students and 47.2% for dentistry students (p=0.69).

FIGURE 1. PREVALENCE OF POOR QUALITY OF SLEEP FOR MEDICINE AND DENTISTRY PROGRAMS, FOR THE TOTAL



* 6th-year students included.

There was no significant difference between good and bad sleep quality between the total number of medicine and dentistry students and between the years of each program. These comparisons were made by logistic regression after adjustments for age, gender, and income, when necessary (Table 1).

For medicine, there was a difference between the mean times of use of computers during the night (p=0.04) and computer (p<0.001) and cell phone use (p<0.001) immediately before sleep, which were greater among students with poor sleep quality. For dentistry, the average time of computer use immediately before sleep (p=0.03) was higher among those with poor sleep quality (p=0.03). The power of all these tests was 100% (Table 2).

Considering only students with poor sleep quality, in the comparison between the programs, dentistry students presented greater averages of cell phone use in 24 hours (p<0.001) and during the night (p<0.001), and medicine students had higher averages of computer use in 24 hours (p=0.01) and immediately before sleep (p=0.01). None of these variables were associated with socioeconomic factors.

TABLE 1. COMPARISON BETWEEN MEDICINE AND DENTISTRY STUDENTS REGARDING THE QUALITY OF SLEEP, IN TOTAL AND FOR EACH YEAR OF EACH PROGRAM.

Variables	Quality of sleep		p*	P (CI 95%)**
	Poor	Good		
	n (%)	n (%)		
All Years			0.79	0.89 (0.63-1.69)
Medicine	127(49.2)	80(47.9)		
Dentistry	131(50.8)	87(52.1)		
First-year			0.35	0.86 (0.24-3.29)
Medicine	22(59.5)	17(48.6)		
Dentistry	15(40.5)	18(51.4)		
Second-year			0.42	0.33 (0.53-6.49)
Medicine	31(51.7)	11(42.3)		
Dentistry	29(48.3)	15(57.7)		
Third-year			0.07	0.49 (0.51-4.05)
Medicine	20(36.4)	15(40.5)		
Dentistry	35(63.6)	22(59.5)		
Fourth-year			0.49	0.89 (0.30-2.82)
Medicine	17(37.8)	12(46.2)		
Dentistry	28(62.2)	14(53.8)		
Fifth-year			0.80	0.71 (0.42-3.58)
Medicine#	37(60.7)	25(58.1)		
Dentistry	24(39.3)	18(41.9)		

* Chi-square

** Values after adjustment by logistic regression. All years (age, gender, and income). First-year (age and income). Second-year (age, gender, and income). Third and fourth years (age and gender). Fifth-year (gender and income).

#6th-year students included.

TABLE 2. COMPARISON OF MEDICINE AND DENTISTRY STUDENTS WITH GOOD AND POOR SLEEP QUALITY REGARDING THE USE OF COMPUTERS AND CELL PHONES.

Variables	Poor Quality of Sleep	Good Quality of Sleep	p**
	Mean (SD) * Median	Mean (SD) * Median	
Medicine			
Use of the computer			
In 24 hours (hours)	2.6 (0.1) 2.0	2.4 (0.2) 2.0	0.24
During the night (hours)	1.9 (0.1) 2.0	1.6 (0.1) 2.0	0.04
Immediately before sleep (minutes)	28.0 (0.33) 15.0	16.2 (3.7) 0.0	<0.001
Use of the cell phone			
In 24 hours (hours)	3.9 (0.3) 3.0	3.1 (0.3) 2.7	0.19
During the night (hours)	1.8 (0.1) 2.0	1.4 (0.1) 1.0	0.09
Immediately before sleep (minutes)	33.3 (2.8) 30.0	17.4 (2.4) 10.0	<0.001
Dentistry			
Use of the computer			
In 24 hours (hours)	2.1 (0.1) 2.0	1.9 (0.1) 2.0	0.35
During the night (hours)	1.7 (0.1) 2.0	1.5 (0.1) 1.2	0.39
Immediately before sleep (minutes)	21.9 (3.1) 0.0	14.4 (3.5) 0.0	0.03
Use of the cell phone			
In 24 hours (hours)	5.3 (0.6) 4.0	6.0 (0.4) 4.0	0.93
During the night (hours)	2.5 (0.1) 2.0	2.7 (0.2) 3.0	0.90
Immediately before sleep (minutes)	40.5 (3.1) 30.0	37.3 (3.5) 30.0	0.33

* SD = standard deviation

**Mann-Whitney Test

DISCUSSION

Poor quality of sleep was a frequent complaint among medicine and dentistry students, with a prevalence of 61.4% and 60.1%, respectively. Similar prevalences (PSQI) were found in medical students from other universities, from the 1st to the 6th year in Paraíba (61.5%)[3] and from the 2nd year in Niterói (64.6%)[2]. There were higher prevalences among medical students (1st to 4th year) of Mogi das Cruzes (84%)[6], from the 1st to the 8th term in Tubarão (76.1%)[4], in a university of India (72.9%)[10], and in 564 women students of dentistry in an university in Saudi Arabia (72.5%)[11]. Whereas in 1st-year medical students of Taiwan, there was a prevalence of 33.8%[12].

A study that included medical students found poor sleep quality in only 19.17% of them in China[13] and in 14.9% of the 234 medical students and 42 medical residents of a University in Goiás. Despite the low percentage, the authors concluded that the group researched slept, on average, a lower number of hours, had greater daytime sleepiness, and made greater use of hypnotic drugs in comparison with the general adult population[1].

It is important to highlight that the prevalence of poor sleep quality can be related to methodological aspects, such as the collection of data in different periods of the year. For example, there was a significant difference in the prevalence of poor sleep quality (PSQI) between periods pre-test (59%), post-test (8%), with no tests (29%)[14]. These results increase the concern with students in this study since they presented a high percentage of poor quality of sleep when there were no tests.

In this study, the mean times of computer use during the night and computer and cell phone use immediately before bedtime among students with poor sleep quality were higher than among those with good quality. Among 710 students from different programs of the University of Minas Gerais, the use of the computer between 19h and 22h or 19h and 24h was associated with a greater frequency of poor quality of sleep (PSQI), with no difference between the groups regarding watching tv and sleep quality[5]. In a University of Mogi das Cruzes, 76 medical students (1st to 4th year) were evaluated regarding their normal use of cell phones and after not using it one hour before bedtime, for 15 days. After the intervention, there were significant increases in the average sleep duration and reduction in the proportion of poor quality of sleep (PSQI), besides a reduction of daytime sleepiness[6]. Whereas with 301 medical students from a university of Tubarão, among other findings, the multivariate analysis showed that the greater the time of internet use per day, the greater the chance of poor sleep quality (PSQI), as well as with the use of YouTube[4].

Similar results were found in other countries. In India, academic females of the 1st year of medicine who used their cell phones for more than 2 hours at night showed a significant correlation between more hours of use and worse quality of sleep (PSQI)[15]. Among 450 students of five universities of medical sciences in Iran, there was a significant correlation between the excessive use of cell phones and the total score of general health and worse quality of sleep (PSQI)[16]. In a study with 350 students of psychology in the United States, there was no correlation between the general use of cell phones and the quality of sleep (Sleep Quality Index). However, the problematic use of cell phones, addictive, problematic, and pathological texting was correlated to a worse quality of sleep[7]. In a university from Turkey, 319 students showed, among other factors, a significant correlation between greater severity of cell phone use (Smartphone Addiction Scale) and global PSQI scores (poorer quality of sleep)[17].

The use of electronic devices that emit light before bedtime prolongs the time to fall asleep, slows the circadian clock, suppresses melatonin levels, reduces the amount and delays the time of REM sleep, and reduces the state of alert the next morning[8].

Generally, when using their cell phones or computers, users are very close to the light emitted by the screen and interact actively with the appliances, which differs from the use of passive technology.

From the findings of this study, there is a need to focus on the sleep care of students related to the use of computers and cell phones in the evening and immediately before bedtime. In addition to sleep hygiene measures, psychological interventions should be considered[18].

The limitations of this study include its cross-sectional nature and the fact that it was carried out in a single institution. Students in the use of psychotropic substances[19] and/or in treatment for mental health problems may have altered quality of sleep. A greater sample number could find differences in the prevalence of poor sleep quality. Despite these limitations, the results can contribute to a better understanding of the quality of sleep and the use of computers and cell phones in the population studied.

CONCLUSION

We observed a high prevalence of poor quality of sleep among students of medicine and dentistry, without difference between them. The mean times of computer use during the night, computer and cell phone use immediately before bedtime (medicine), and the computer immediately before bedtime(dentistry) were higher among students with poor sleep quality were higher than among those with good quality.

Conflicts of interest

The authors declare there are no conflicts of interest.

Contribution of the authors:

Diogo von Gaevernitz Lima and Ana Claudia Garabeli Cavalli Kluthcovsky: design, methodology, analysis, interpretation of data, drafting of the manuscript, and critical review.

Luiz Gustavo Rachid Fernandes and Giovane Okarenski: methodology, data interpretation, manuscript drafting, and critical review.

RESUMO

OBJETIVO: *Avaliar a qualidade de sono e sua associação com uso de computadores e celulares em estudantes de medicina e odontologia.*

MÉTODOS: *Estudo transversal e comparativo, que avaliou 425 estudantes por meio de questionário socioeconômico, Índice de Qualidade do Sono de Pittsburgh (PSQI) e uso de computador e telefone celular.*

RESULTADOS: *Sono de má qualidade foi observado em 61,4% dos estudantes de medicina e em 60,1% de odontologia. Para os estudantes de medicina, os tempos médios de uso de computador durante a noite (p=0,04) e computador (p<0,001) e celular (p<0,001) imediatamente antes de dormir foram maiores para os estudantes com má qualidade de sono. Para os estudantes de odontologia, o tempo médio de uso do computador imediatamente antes de dormir foi maior para aqueles com má qualidade de sono (p=0,03).*

CONCLUSÃO: *Os estudantes devem receber orientações sobre estratégias de prevenção e cuidados com a qualidade do sono.*

PALAVRAS-CHAVE: *Sono. Estudantes de medicina. Estudantes de odontologia. Tecnologia.*

REFERENCES

1. Cardoso HC, Bueno FCC, Mata JC, Alves APR, Jochims I, Vaz Filho IHR, et al. Avaliação da qualidade do sono em estudantes de Medicina. Rev Bras Educ Med. 2009;33(3):349-55.
2. Pagnin D, Queiroz V, Carvalho YT, Dutra AS, Amaral MB, Queiroz TT. The relation between burnout and sleep disorders in medical students. Acad Psychiatry. 2014;38(4):438-44.
3. Rique GL, Fernandes Filho GM, Ferreira AD, Sousa-Muñoz RL. Relationship between chronotype and quality of sleep in medical students at the Federal University of Paraiba, Brazil. Sleep Sci. 2014;7(2):96-102.
4. Marin CE, Feldens VP, Sakae TM. Dependência de internet, qualidade do sono e sonolência em estudantes de Medicina de Universidade do Sul do Brasil. Rev AMRIGS. 2016;60(3):191-7.
5. Mesquita G, Reimão R. Quality of sleep among university students: effects of nighttime computer and television use. Arq Neuropsiquiatr. 2010;68(5):720-5.
6. Freitas CCM, Gozzoli ALDM, Konno JN, Fues VLR. Relação entre uso do telefone celular antes de dormir, qualidade do sono e sonolência diurna. Rev Med. 2017;96(1):14-20.
7. White AG, Buboltz W, Igou F. Mobile phone use and sleep quality and length in college students. Int J Humanit Soc Sci. 2011;1(18):51-8.
8. Chang AM, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. Proc Natl Acad Sci U S A. 2015;112(4):1232-7.
9. Bertolazi AN, Fagondes SC, Hoff LS, Dartora EG, Miozzo IC, Barba ME, et al. Validation of the Brazilian Portuguese version of the Pittsburgh Sleep Quality Index. Sleep Med. 2011;12(1):70-5.
10. Shad R, Thawani R, Goel A. Burnout and sleep quality: a cross-sectional questionnaire-based study of medical and non-medical students in India. Cureus. 2015;7(10):e361.
11. Elagra MI, Rayyan MR, Alnemer OA, Alshehri MS, Alsaffar NS, Al-Habib RS, et al. Sleep quality among dental students and its association with academic performance. J Int Soc Prev Community Dent. 2016;6(4):296-301.
12. Kang JH, Chen SC. Effects of irregular bedtime schedule on sleep quality, daytime sleepiness, and fatigue among university students in Taiwan. BMC Public Health. 2009;9:248.
13. Feng GS, Chen JW, Yang XZ. Study on the status and quality of sleep-related influencing factors in medical college students. Zhonghua Liu Xing Bing Xue Za Zhi. 2005;26(5):328-31.
14. Ahrberg K, Dresler M, Niedermaier S, Steiger A, Genzel L. The interaction between sleep quality and academic performance. J Psychiatr Res. 2012;46(12):1618-22.
15. Yogesh S, Abha S, Priyanka S. Mobile usage and sleep patterns among medical students. Indian J Physiol Pharmacol. 2014;58(1):100-3.
16. Eyvazlou M, Zarei E, Rahimi A, Abazari M. Association between overuse of mobile phones on quality of sleep and general health among occupational health and safety students. Chronobiol Int. 2016;33(3):293-300.
17. Demirci K, Akgönül M, Akpinar A. Relationship of smartphone use severity with sleep quality, depression, and anxiety in university students. J Behav Addict. 2015;4(2):85-92.
18. Friedrich A, Schlarb AA. Let's talk about sleep: a systematic review of psychological interventions to improve sleep in college students. J Sleep Res. 2018;27(1):4-22.
19. Finger G, Silva ER, Falavigna A. Use of methylphenidate among medical students: a systematic review. Rev Assoc Med Bras. 2013;59(3):285-9.

ORIGINAL ARTICLE

Evaluation of KI-67 expression in uterine leiomyoma and in healthy myometrium: a pilot study

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SUMMARY

OBJECTIVE: *Evaluate the expression of KI-67 in uterine leiomyomas and adjacent myometrial tissue and verify the existence of a correlation between clinical parameters and KI-67 expression in tumors.*

METHODS: *This is a cross-sectional, controlled, analytical study. Samples of leiomyomas and myometrium were obtained from patients who underwent hysterectomy. The samples were processed by immunohistochemistry using KI-67 antibody, and the expression was evaluated by two blinded observers. Student›s T-test was used for comparison of means, and Pearson›s P test for correlation with clinical parameters.*

RESULTS: *A total of 9 patients were included in the study. The mean age was 40.7 years, ranging from 35 to 44 years. The mean expression of KI-67 in myometrium was 1.63%, and, in leiomyomas, 5.96% (p <0.001). The highest expression of KI-67 was moderately related to the severity of anemia, bleeding, and pain level.*

CONCLUSION: *The expression of KI-67 in normal myometrium was significantly lower than in leiomyomas. The highest expression of KI-67 was moderately related to the severity of anemia, bleeding, and pain level in the patients of this study.*

KEYWORDS: *Ki-67 Antigen. Leiomyoma. Myometrium.*

INTRODUCTION

Uterine fibroids or leiomyomas are benign tumors that originated from the smooth muscle of the uterus. Their estimated incidence reaches 75% of childbearing-age women. They are characterized histologically by areas of disordered growth of smooth muscle fibers and extracellular matrix[1]. These tumors arise naturally during the reproductive age. They are usually related to biological changes in growth and

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development mainly influenced by sex hormones, especially estrogen, in addition to other causes such as genetic changes and growth factors[1].

Leiomyomas can have a significant impact on the quality of life of women in reproductive age[2]. Depending on their anatomical position, quantity, and size, these tumors can cause menstrual irregularity in about 30% of cases, with hemorrhage being the most frequent symptom. Increased uterine volume may induce an elevated pelvic pressure, leading to pain and compression of other structures, such as the rectum and bladder, causing constipation and urinary incontinence. Moreover, uterine fibroids have a negative impact on reproductive function and are associated with infertility and adverse gestational outcomes, such as miscarriages, fetal anomalies, premature births, and an increase of indications for cesarean sections[3].

Due to its high prevalence in the population and its impact, its pathogenesis must be fully understood in order to develop better therapeutic strategies. The aim of this study was to evaluate the expression of the KI-67 cell proliferation marker in uterine leiomyomas and adjacent myometrial tissue. Furthermore, this paper aims to verify the existence of a connection between clinical parameters and KI-67 expression in tumors.

METHODS

Type of study and sample design

This study is part of a research project whose goal is to evaluate several markers (KI-67, BCL-2, IGF-1, among others) and their clinical correlations in the symptomatology of uterine leiomyomatosis. A significant sample size of 60 was determined based on the number of patients treated at our service. Because this is a pilot study, it was decided to reduce the sample to 10 to analyze the viability of the research. In addition, we selected KI-67 as the first marker to be studied due to the greater experience of the pathology team with this marker. In addition, its qualitative analysis is simpler when compared to the others.

This is a cross-sectional, controlled, and analytical study. This study included ten women with symptomatic uterine leiomyomatosis who underwent a total abdominal hysterectomy at a tertiary hospital in Teresina-PI. They were randomly selected by batch from March 2017 to December 2017. The inclusion criteria were: women over 18 years of age with symptomatic uterine leiomyomatosis and a surgical indication of a total hysterectomy by laparotomy. The exclusion criteria were: menopausal women, patients with a cancer diagnosis or with clinical suspicion of malignancy, previous hormone therapy or surgical intervention for uterine fibroids.

Collection of samples

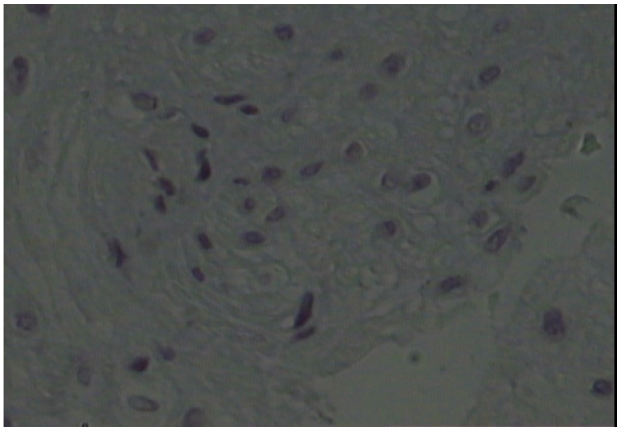
After the hysterectomy, two tissue samples were collected of one-centimeter diameter. One sample was composed of uterine leiomyoma and the other one of myometrial tissue. The healthy myometrium was located at least five centimeters away from any leiomyoma. Then, the samples were submitted to the following procedures: 1) Formalin fixation; 2) Dehydration through ethyl alcohol; 3) Diaphonization in xylol; 3) Impregnation with paraffin at an oven temperature of 59 ° C. After this process, part of the samples underwent the consecutive serial cuts of four micrometers. Next, they were kept on glass slides and stained with hematoxylin and eosin for diagnostic confirmation of normal myometrial tissue (figure 1) and uterine leiomyomatosis (figure 2).

In addition, the following data were collected from medical records: age, gestational age, fibroids with greater volume, uterine volume, intensity of menstrual bleeding (mild, moderate, or severe), post-operative hemoglobin, Body Mass Index (BMI) and ethnicity. Due to the intense menstrual irregularity that some participants presented, it was not possible to accurately assess their stage of the menstrual cycle.

Immunohistochemical method

A monoclonal antibody to KI-67 was used. The samples were dewaxed in xylol at 110 ° C and, soon after, subjected to multiple washouts with water at

FIGURE 1. HEALTHY MYOMETRIUM (400X MAGNIFICATION)



room temperature. After this, the cuts were placed in ethyl alcohol at concentrations 100, 80, and 50 percent consecutively. Then, they were washed in running tap water. Lastly, the sections underwent distillation. Endogenous peroxidase activity was blocked with hydrogen peroxide (H2O2) 3% three times for 10 minutes each. This last step was followed by washing the samples with Phosphate-Buffered Saline (PBS) solution (pH 7.4 to 7.6). In order to unmask antigens, the slides were boiled at 95°C for 30 minutes in sodium citrate buffer solution (pH 6.0) in a T-fal streamer. After cooling for 20 minutes, the sections were washed in tap and distilled water and, ultimately, placed in PBS. The incubation time with a specific primary antibody was overnight at 4° C.

After incubation, the slides were washed three times in PBS, dried and incubated with «EnVisionTM System (DAKO, Code K 1672)» for 1 hour at 37 ° C. Once again, the sections were washed in tap and distilled water and then stained with Mayer›s hematoxylin for 30 seconds. The cuts underwent alcohol-xylol dehydration and, lastly, placed on coverslips with Entellan resin.

Samples evaluation

The expression of the biomarkers was evaluated by two blinded observers with no information regarding sample identification. These observers counted the number of cells with positively stained nuclei under 400x magnification using an optical microscope attached to a video camera.

For the quantification of KI-67 biomarker expression, 500 cells were counted on each slide. The percentage of stained cells was calculated from the ratio of the number of cells with stained nuclei and the total number of cells multiplied per 100, as in the formula below:

Percentage of stained cells = (cells with colored nuclei x 100)/Total number of cells

Statistical analysis

The data were tabulated using Microsoft Excel 16.0 and summarized in tables and graphs; Student›s t-test was used for statistic comparisons. The significance level was set at $p<0.05$. Pearson›s correlation coefficient was used for paralleling the clinical findings and the KI-67 expression according to the following parameters:

0.9 to 1.0 indicates a very high correlation.
0.7 to 0.9 indicates a high correlation.
0.5 to 0.7 indicates a moderate correlation.
0.3 to 0.5 indicates a low correlation.
0 to 0.3 indicates a negligible correlation.

Ethical aspects

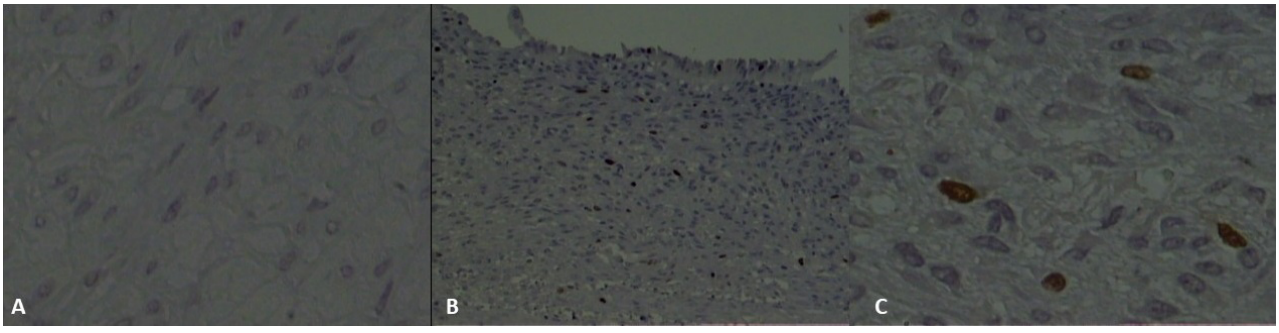
The research was approved by the Research Ethics Committee of our institution under protocol number 2.061.409. All patients signed an informed consent form.

RESULTS

A total of 9 patients were included in the study because one of the samples was not considered satisfactory. The mean age was 40.7 years, ranging from 35 to 44 years. Regarding ethnicity, eight patients were African American, and one was white. The mean size of fibroids was 5.4 cm, ranging from 2.1 to 10.5 cm. The mean uterine volume was 345 cm3 ranging from 127 to 449 cm^3.

Three (33.3%) patients classified the pain as mild, and six (66.7%) as severe. Bleeding was considered mild in three (33.3%) patients and intense in the remainder (66.7%). Only three patients had preoperative

FIGURE 2. PANEL A: UTERINE LEIOMYOMA (400X MAGNIFICATION). PANEL B: UTERINE LEIOMYOMA WITH CELLS EXPRESSING KI-67 (40X MAGNIFICATION). PANEL C: UTERINE LEIOMYOMA WITH CELLS EXPRESSING KI-67 (400X MAGNIFICATION)



hemoglobin lower than nine; in one case, it was necessary to perform a perioperative blood transfusion. Two patients were nulliparous, two had two gestations, and five became pregnant three or more times. The mean BMI was 24.2, ranging from 21.3 to 29.1; therefore, five patients had adequate BMI (19-24.9), and the remainder were overweight.

Regarding KI-67 expression, there were positively stained cells in all samples included (Figure 2). The mean expression of KI-67 in the myometrium was 1.63%, and, in leiomyomas, it was 5.96% ($p < 0.001$). The correlation between clinical parameters and KI-67 expression in leiomyomas is presented in Table 1.

TABLE 1. CORRELATION BETWEEN KI-67 EXPRESSION IN UTERINE LEIOMYOMAS AND CLINICAL PARAMETERS ACCORDING TO PEARSON'S CORRELATION COEFFICIENT.

Variable	P
Age	-0.46
Parity	-0.04
Tumor size	0.12
Uterine volume	0.21
Pain	0.55
Bleeding	0.55
Preoperative hemoglobin	-0.55
Body Mass Index (BMI)	0.05
Weight	0.1

DISCUSSION

Uterine leiomyomas are benign neoplasms of the smooth muscle of the uterus. They are usually asymptomatic, although they may manifest as abnormal uterine bleeding, pelvic pain, and various combinations of other symptoms that may impair the quality of life of women. They account for 30% of hysterectomies in women during reproductive age and 9.4 billion dollars in medical expenses in the United States[4].

In recent years, there has been a significant advance in the knowledge of the biology of leiomyomas, but the fundamental mechanisms of their formation are not yet fully understood. More than 200 altered genes have been reported in these tumors, although a few remain prevalent in several populations[1]. Several signaling pathways may also be altered, reflecting the complexity of this neoplasm. It is believed that the probable origin of this neoplasm derives from injuries and successive repairs of the myometrium. Therefore, we can state that, initially, non-hormonal factors are responsible for the onset of the myoma. In a second moment, estrogen and progesterone would play their role in allowing tumor growth[1].

Classically, leiomyomas are described more frequently in African American women. In addition, the symptomatology is more intense in this group. Other contributors, such as nulliparity, use of progesterone-based contraceptives, obesity, hypertension, and early menarche, have also been associated as risk factors for this disease. On the other hand, the use of combined contraceptives and a diet rich in vegetables seem to exert a protective effect[4,5]. In fact, some of these agents do not have their mechanisms of action completely clarified, but they can act by deregulating specific pathways, allowing a greater cellular proliferation[1,4,5].

KI-67 is a biomarker that has been researched for years in oncology. It is used to assess the degree of proliferation of a given tissue. When the nucleus of the cell binds to the antibody and produces a characteristic coloration, it indicates that the cell is advancing in the cell cycle. This marker is useful because it allows evaluating the aggressiveness of a tumor: the higher the number of positive cells, the higher the cell proliferation index. Thus, tumors with a greater expression of KI-67 are classically described as more aggressive[6,7].

At this moment, a few studies have focused on the expression of KI-67 in fibroids. They usually focus on using it only as a reference to distinguish fibroids from malignant neoplasms. As a result, it is not known how the greater or lesser expression of this marker may influence the presentation or prognosis of uterine leiomyomatosis. The authors concluded that the rate of cell proliferation is lower in myomas than in uterine sarcomas, which is a useful parameter in cases of unclear diagnoses[8-10].

In our study, KI-67 expression was significantly higher in fibroids than in healthy myometrium. In addition, we found a moderate correlation between KI-67 expression and the reported severity of anemia, level of pain, and bleeding. The value of 0.55 for the correlation between KI-67 expression, pain, and bleeding suggests that the greater the expression of the marker, the greater the intensity of these clinical parameters. Even though the values of correlation of this study are not considered high

(0,7 – 1), they are calculated in a small sample size, suggesting that there is an important relationship among the data studied in this article. Comparatively, the value of - 0.55 calculated for the correlation between KI-67 and preoperative hemoglobin indicates that the higher the marker expression, the lower the level of hemoglobin. The presence of a negative correlation between these two parameters is probably due to the greater bleeding intensity in patients with higher KI-67 expression.

Due to the low number of cases included in this initial study, it is still very early to state that the cell proliferation index is a prognostic factor to be incorporated into clinical practice. However, the results suggest that it probably relates to higher symptomatology. Furthermore, we can conclude that the methodology of this article can be applied to analyze a large number of patients and that the sample size should be increased in order to improve the statistical analysis.

CONCLUSION

The expression of KI-67 in normal myometrium was significantly lower than in leiomyomas. The highest expression of KI-67 was moderately related to the severity of anemia, bleeding, and pain level in the patients of this study.

Author Contributions:

Concept – Walberto Monteiro Neiva Eulálio Filho, Benedito Borges Silva, Pedro Vitor Lopes Costa ; Design – Maria Simone Oliveira Lima, Rodolfo Myronn de Melo Rodrigues; Supervision – Eduardo Augusto Soares Sousa, Benedito Borges Silva; Resources – All authors; Materials – Emerson Davi do Nascimento Brazil, Pedro Vitor Lopes Costa; Data Collection and/or Processing – Walberto Monteiro Neiva Eulálio Filho, Benedito Borges Silva, Eduardo Augusto Soares Sousa; Analysis and/or Interpretation – All authors ; Literature Search – All authors; Writing Manuscript – All authors ; Critical Review – All authors; Final Review – All authors.

RESUMO

OBJETIVO: *Avaliar a expressão do KI-67 em leiomiomas uterinos e tecido miometrial adjacente e verificar a existência de correlação entre parâmetros clínicos e expressão do KI-67 em tumores.*

MÉTODOS: *Estudo transversal, controlado e analítico. Amostras de leiomiomas e miométrio foram obtidas de pacientes que realizaram histerectomia. As amostras foram processadas por imuno-histoquímica utilizando anticorpo para KI-67 e a expressão avaliada por dois observadores cegos. O teste t de Student foi utilizado para comparação de médias e o teste P de Pearson para correlação com parâmetros clínicos.*

RESULTADOS: *Um total de 9 pacientes foi incluído no estudo. A idade média foi de 40,7 anos, variando de 35 a 44 anos. A expressão média do KI-67 no miométrio foi de 1,63% e nos leiomiomas de 5,96% (p <0,001). A maior expressão do KI-67 foi moderadamente relacionada com a gravidade da anemia, sangramento e nível de dor.*

CONCLUSÃO: *A expressão do KI-67 no miométrio normal foi significativamente menor que nos leiomiomas. A maior expressão do KI-67 foi moderadamente relacionada à gravidade da anemia, sangramento e nível de dor nos pacientes deste estudo.*

PALAVRAS-CHAVE: *Antígeno KI-67. Leiomioma. Miométrio.*

REFERENCES

1. Commandeur AE, Styer AK, Teixeira JM. Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. Hum Reprod Update. 2015;21(5):593-615.
2. Vargas-Hernández VM, Vargas-Aguilar VM, Tovar-Rodríguez JM, Flores-Barrios K, Acosta-Altamirano G, Moreno-Eutimio MA. Leiomiomatosis uterina. Aspectos epidemiológicos, fisiopatogénicos, reproductivos, clínicos y terapêuticos. Rev Hosp Jua Mex. 2013;80(3):173-82.
3. Boclin KLS, Faerstein E. Prevalência de diagnóstico médico auto-relatado de miomas uterinos em população brasileira: padrões demográficos e socioeconômicos no Estudo Pró-Saúde. Rev Bras Epidemiol. 2013;16(2):301-13.
4. Wise LA, Laughlin-Tommaso SK. Epidemiology of uterine fibroids: from menarche to menopause. Clin Obstet Gynecol. 2016;59(1):2-24.
5. Styer AK, Rueda BR. The epidemiology and genetics of uterine leiomyoma. Best Pract Res Clin Obstet Gynaecol. 2016;(34):3-12.
6. Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, et al. An international Ki67 reproducibility study. J Natl Cancer Inst. 2013;105(24):1897-906.
7. Dowsett M, Nielsen TO, A›Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2011;103(22):1656-64.
8. Petrović D, Babić D, Forko JI, Martinac I. Expression of Ki-67, P53 and progesterone receptors in uterine smooth muscle tumors. Diagnostic value. Coll Antropol. 2010;34(1):93-7.
9. Stănescu AD, Nistor E, Sajin M, Stepan AE. Immunohistochemical analysis in the diagnosis of uterine myometrial smooth muscle tumors. Rom J Morphol Embryol. 2014;55(3 Suppl):1129-36.
10. Mills AM, Ly A, Balzer BL, Hendrickson MR, Kempson RL, McKenney JK, et al. Cell cycle regulatory markers in uterine atypical leiomyoma and leiomyosarcoma: immunohistochemical study of 68 cases with clinical follow-up. Am J Surg Pathol. 2013;37(5):634-42.

ORIGINAL ARTICLE

Comparison of quality of life and functionality in type 2 diabetics with and without insulin

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SUMMARY

INTRODUCTION: *Type 2 diabetes mellitus compromises physical, psychological, economic, and social life.*

OBJECTIVES: *To identify and compare the quality of life, depression, functional performance, and physical activity in patients with type 2 diabetes mellitus who use insulin or not.*

METHODS: *A observational, descriptive, cross-sectional, comparative study involving 100 patients (50 use insulin and 50 do not) from a Teaching Hospital. Questionnaires used: Identification and Socioeconomic Profile; SF-36; Hospital Anxiety and Depression Scale; Visual Analogue Scale for Pain; Canadian Occupational Performance Measure, and International Physical Activity Questionnaire.*

RESULTS: *Sample composed predominantly by middle-aged, female, married, retired, and with incomplete elementary school individuals. There is impairment in all domains of quality of life, being more intense in functional capacity, physical limitations, pain, social aspects, limitation by emotional aspects, and mental health (P<0.05). There is a significant prevalence of anxiety or depressive symptoms in the groups, especially in those using insulin. However, the occurrence of the corresponding psychiatric disorders is unlikely (P<0.05). There was no significant difference in neuropathic pain between the groups (P=0.2296). Functional impairment is similar in both groups regarding self-care activities (P=0.4494) and productivity (P=0.5759), with a greater deterioration of leisure time in patients on insulin (P=0.0091). Most of them practice physical activity, predominantly walking, with no significant difference when comparing the groups (P>0.05), as well as in the other modalities.*

CONCLUSION: *Insulinized patients present greater impairment of functional capacity and socialization, as well as greater neuropathic pain, anxiety, and depressive symptoms.*

DESCRIPTORS: *Diabetes mellitus, type 2. Insulin. Quality of life. Mental health. Occupational therapy. Exercise.*

INTRODUCTION

Diabetes mellitus (DM) is a chronic non-transmissible disease, resulting from the inability to produce and/or the non-use of endogenous insulin, which causes hyperglycemia and, consequently, abnormalities in glucose, lipid, and protein metabolism.[1,2] According to the International Diabetes Federation,

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there are 415 million diabetics in the world, 14 million in Brasil, of which 90% are type 2, 5-10% type 1, and 2% are other types or associated syndromes.[2-4] There is an estimated increase of 50% in its prevalence in the next 20 years, and its main risk factors are: female gender, age, high body mass index (BMI), systemic arterial hypertension (SAH), and family history of diabetes.[5,6]

This disease generates profound impacts related to complications that can be acute (hypoglycemia, ketoacidosis, and hyperosmolar coma) or chronic (cardiovascular disease, renal insufficiency, amputation of limbs, neuropathy, nephropathy, and retinopathy).[7] Although mortality due to complications has decreased, the quality of life and well-being of diabetic individuals are still very much affected, with changes in work, family, sexuality, physical, social, and emotional well-being, physical changes, and the significance of these changes.[1]

After the diagnosis, it is common to react with anger, revolt, and depression due to biological, psychological, and social changes that influence acceptance and adherence to the treatment of the disease since it requires self-care (adopt healthy behaviors) and the use of long-term medications to avoid complications, thus making the prognosis of type 2 diabetes mellitus (DM2) unfavorable.[8]

Mild/moderate physical exercise increases insulin sensitivity and the muscle uptake of glucose, reduces body fat, improves the heart frequency, and the lipid and glycemic control, among others, in all individuals, promoting physical and mental well-being.[9]

In DM2, often the mere change of lifestyle is not enough; therefore, the combined treatment with medications (oral hypoglycemic agents and/or insulin) aims to achieve levels of glycated hemoglobin (Hba1c) lower than 7% in order to reduce the risk of micro and macrovascular complications.[10]

Several factors influence the quality of life of diabetic individuals, such as the use of insulin, age, chronic complications, socioeconomic and educational level, psychological factors, knowledge about the disease, and care received. This study will evaluate the different influences of the treatment used - oral hypoglycemic agents or insulin - in quality of life, depression, functional performance, and physical activity in patients with DM2, enabling the development of strategies and intervention programs to promote integral care to diabetic patients.

METHODS

This is an individual, observational, descriptive, cross-sectional, comparative study carried out at the Outpatient Clinic of Endocrinology of the Hospital de Base in São José do Rio Preto, from August 2016 to April 2017, approved by the Research Ethics Committee of Famerp, decision No. 1,575,216. A total of 100 DM2 patients were included - 50 in the Hypoglycemic Drugs Group- MG - and 50 in the Insulin Group - IG. They were approached while waiting for their medical consultations and agreed to participate by signing the Informed Consent Form.

The participants are patients with a confirmed diagnosis of DM2, who use medications or insulin, aged over 18 years, regardless of sex, socioeconomic conditions, and ethnicity enrolled from a convenience sample.

These patients responded to six questionnaires: Identification Sheet and Socioeconomic Profile; SF-36 Inventory of Quality of Life, grading the general state of health; Hospital Anxiety and Depression Scale - HADS, evaluating, separately, anxiety (HADS-A) and depression (HADS-D); the Canadian Occupational Performance Measure - COPM, analyzing the functional capacity; Visual Analogue Scale of Pain - VAS, measuring the intensity of pain; and International Physical Activity Questionnaire - IPAQ, reporting the frequency and duration of physical activities based on the previous week.

Using techniques of descriptive and inferential statistics, we calculated absolute frequencies, percentages, measures of central tendency, and dispersion. We used the Kolmogorov-Smirnov normality test or Fischer's Test and, subsequently, for comparison between groups, the Student's t-test or Mann-Whitney test. A p-value < 0.05 was considered statistically significant. The software used was Graphpad Instat 3.10 (2009).

RESULTS

After analyzing the socioeconomic data, we found an average age of 56.12±13.78 years and the average time of diagnosis of 10.46 years in the MG and 59.46±11.48 years and 12.86 years in the IG, respectively. In our sample, most individuals were female, married, with incomplete basic education, and retired.

The SF-36 Inventory of Quality of life (Table 1), comprising eight scales, scored from 0 to 100, shows a better quality of life, the higher the score obtained. Among diabetic individuals, all areas were affected,

in different intensities individually and per domain, with an statistical significance in functional capacity (P=0.0002), limitation due to physical aspects (P=0.0002), pain (P=0.0076), social aspects (P<0.0001), limitation due to emotional aspects (P<0.0001) and mental health (P<0.0001), indicating a greater impairment in the IG.

The analysis of the Hospital Anxiety and Depression Scale - HADS (Figure 1) indicated the presence of anxiety or depression symptoms in a large portion of participants in both groups, but anxiety disorders or depression are unlikely. We can also conclude that individuals in the IG have a significantly higher incidence of anxiety (P<0.0001) or depression (P=0.0001).

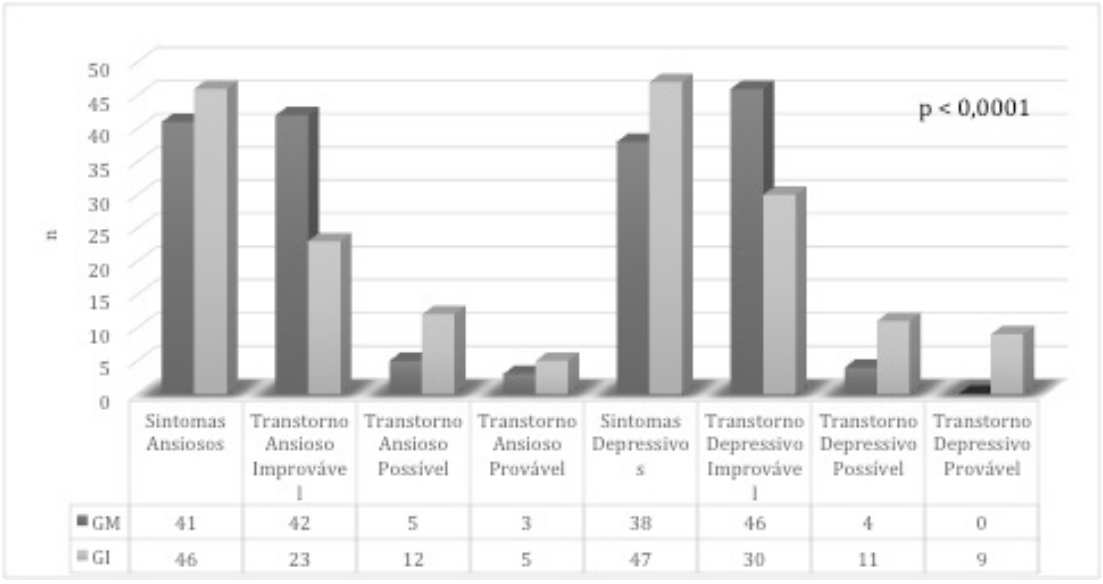
The intensity of neuropathic pain, as assessed by Visual Analog Scale of Pain - VAS, showed, in the MG, a mean ± SD of 4.5 ± 4.036; mode 0; median 5 and maximum and minimum scores of 0 and 10, respectively,

TABLE 1. STATISTICAL DESCRIPTION OF THE SF-36 INVENTORY OF QUALITY OF LIFE SCORE IN EACH DOMAIN OF THE MG AND IG GROUPS

Domain score		Minimum	Maximum	Median	Mean ± SD	P
Functional capacity	MG	35	100	85	83.6 ± 16.84	0.0002
	IG	5	100	67.5	59.9 ± 31.72	
Limitation due to physical aspects	MG	0	100	75	66 ± 35.26	0.0002
	IG	0	100	0	35.5 ± 43.76	
Pain	MG	0	100	61	61.59 ± 32.11	0.0076
	IG	0	100	40	43.32 ± 32.49	
Overall health condition	MG	25	67	52	50.28 ± 10.22	0.0844
	IG	0	100	41	45.03 ± 27.01	
Vitality	MG	20	85	55	54.6 ± 14.06	0.0929
	IG	0	100	45	44.7 ± 27.81	
Social aspects	MG	50	100	100	91 ± 12.88	<0.0001
	IG	0	100	50	54 ± 37.15	
Limitation due to emotional aspects	MG	33.4	100	100	78.68 ± 29.13	<0.0001
	IG	0	100	33.33	52.65 ± 42.10	
Mental health	MG	56	96	80	77.68 ± 10.44	<0.0001
	IG	0	100	60	58.56 ± 25.28	

SD - Standard deviation. MG - Group who used hypoglycemic medication. IG - Group who used insulin

FIGURE 1. HOSPITAL ANXIETY AND DEPRESSION SCALE (HADS) OF DM2 PATIENTS USING MEDICATION (MG) OR INSULIN (IG)



and 62% (31) of these participants presented NPP, with 16% (n=8) reporting living with the worst pain ever experienced (score 10). Regarding the IG, the mean ± SD obtained was 5.58 ± 3.703; mode 0; median 5 and maximum and minimum scores of 0 and 10, respectively; in this group, 74% (n=37) presented NPP, with 16% (n=8) reporting living with the worst pain ever experienced. Therefore, there was no significant difference in NPP between both groups (P=0.2296).

The Canadian Occupational Performance Measure - COPM (Table 2) analyzed the impairment of functionality in self-care, productivity, and leisure activities; independence outside the home, domestic activities, and active recreation were the activities most affected in each class, respectively, in both groups. After comparing the results of the MG and the IG, no statistical significance was found regarding impairment of self-care activities (P=0.4494) and productivity (P=0.5759), whereas in relation to leisure, the greatest impairment in the IG was significant (P=0.0091), highlighting an important impairment of socialization.

Finally, the International Physical Activity Questionnaire - IPAQ showed that 76% of individuals of the MG and 68% of the IG practice physical activity, and walking was the modality with most practitioners in the MG, with 42%, and in the IG, with 52%. However, when comparing both groups, there was no significant difference in adherence to this practice (P=0.1636), but there was in the following modalities: moderate activities, practiced by 22% of members in the MG and 44% in the IG (p = 0.0696); and vigorous activities, with only 2% of practitioners in the MG and 6% in the IG (P=0.3248).

DISCUSSION

In this study, the mean age of participants was greater than 55 years, with a predominance of females, married, with incomplete basic education, and retired, which is in line with other studies.[11]

The SF36 - Inventory of Quality of Life scores indicate that the areas most affected - worst scores - are in the MG, general health state (50.28), vitality (54.6), and pain (61.59), in comparison with physical aspects (35.5), pain (43.32) and vitality (44.7) in the IG. In spite of equivalences in two domains, the GI, with lower averages, presented greater impairment. The best domains were social aspects (91), functional capacity (83.6), and limitation due to emotional aspects (77.68) in the MG, and functional capacity (59.9), mental health (58.56), and social aspects (54) in the IG. We also found, based on the means, equivalence in two areas, but there is a greater impairment in the IG. The lowest scores of the IG are consistent with the literature, due to the influence of therapy in the patient's quality of life.[12]

We conclude by examining the emotional state of the participants, a high presence of anxiety and/or depression symptoms in both groups; however, it is not possible to say that those represent a diagnosis of depressive and/or anxiety disorder. Despite this, the probability of symptoms and/or presence of these disorders is significantly greater among individuals in the IG, which is consistent with previous studies and correlates with more advanced disease and more comorbidities and/or complications, which have a direct emotional impact and do not derive directly from insulin.[13,14] The emotional state is directly related

TABLE 2. DIFFICULTIES REPORTED BY THE PARTICIPANTS IN THE USE OF MEDICATION (MG) AND INSULIN (IG) ACCORDING TO THE CANADIAN OCCUPATIONAL PERFORMANCE MEASURE - COPM

	MG		IG		
Activity	n	%	n	%	P
Self-Care					
Personal Care	2	4	2	4	0.4494
Functional Mobility	3	6	10	20	
Independence away from home	10	20	14	28	
Productivity					
Work	11	22	10	20	0.5759
Domestic Activities	17	34	11	22	
Leisure activities					
Calm Recreation	6	12	4	8	
Active recreation	7	14	18	36	0.0091
Socialization	1	2	16	32	

MG - Group who used hypoglycemic medication. IG - Group who used insulin

to the quality of life, interfering in adherence to treatment and other activities beneficial to health.[13,15,16]

Neuropathic pain (NPP), present in both groups with no statistically significant differences in prevalence in the Mg and the IG, differs from previous studies, which showed a higher occurrence of NPP in type 2 diabetic patients from the MG, because those on insulin (IG) get lower blood glucose levels, thus preventing the development and progression of microvascular complications.[17] The involvement of a large number of the patients studied (62% in the MG and 74% in the IG) also differ in relation to other analyses with a lower percentage.[18,19] The discrepancy in the prevalence of diabetic neuropathy has been reported in some studies, and its possible causes include the age of the population studied, time of diagnosis, control of blood glucose levels, the presence of peripheral arterial disease, and the diagnostic methods employed.[19,20] There is also interference of the diagnostic instrument, because, through the Visual Analogue Scale of Pain - VAS, patients graded their pain intensity. It is known that comorbidities are confounding factors, because patients may report pain without this necessarily being neuropathic, thus raising results of prevalence, which is an important limitation of the present study.[18-20]

The functional capacity of the participants was evaluated by the Canadian Occupational Performance Measure - COPM and indicated commitment in self-care activities, productivity, and leisure, the latter with greater impairment in the IG. Similar results have been observed in other studies.[21] Therefore, DM2 influences functional capabilities, and hyperglycemia, the time of evolution of the disease, and the treatment in use can be aggravating factors.[22,23]

The International Physical Activity Questionnaire - IPAQ revealed that the participants are predominantly active, without statistically significant differences when comparing the practice of physical exercises in both groups; the less intense modalities - walking - were preferred; adhesion progressively decreases with the increase of activity intensity. This behavior differs from the one described in the literature regarding the percentage of diabetic patients adherents to physical activities; however, it converges regarding the type of activity practiced. Thus, the use of strategies that are simple, inexpensive, and of rapid implementation by a health professional is important and has an impact on the behavior related to physical activity in this population.[24,25]

CONCLUSION

Diabetes mellitus, a disease caused by the lack of production or difficulty in using endogenous insulin, generates hyperglycemia and metabolic abnormalities, causing loss of functional capacity and quality of life due to the development of peripheral neuropathic pain, retinopathy, nephropathy, anxiety, depression, and changes in lifestyle. Thus, emphasis should be given to the importance of physical exercises for the treatment of endocrinopathy and emotional changes.

In this study, we found that the patients on insulin have, in general, greater impairment of quality of life, functional capacity, and socialization, and report greater neuropathic pain and anxious and depressive symptoms in relation to those who use medications.

These outcomes are explained by the greater physical and emotional discomfort from the use of insulin for blood glucose control, a more advanced stage of the disease, and a greater presence of comorbidities. Therefore, we must develop strategies and intervention programs to promote the integral care of patients with DM2, from the time of diagnosis, to prevent the progression of the disease and its complications, considering the physical and emotional impact of each therapeutic option, and not just the indications based on the control of blood glucose levels.

Contribution of the authors

Ana Carolina Reis – Concept of the project, data collection and analysis, reference search, and drafting of the article.

Milena Vizioli Cunha – Concept of the project, data collection and analysis, reference search, and drafting of the article.

Maysa Alahmar Bianchin – Advisor.

Maristella Tonon Rui Freitas – Advisor.

Lilian Castiglioni – Statistical analysis.

RESUMO

INTRODUÇÃO: *Diabetes mellitus tipo 2 compromete física, psicológica, econômica e socialmente.*

OBJETIVOS: *Identificar e comparar qualidade de vida, depressão, desempenho funcional e exercício físico em pacientes com diabetes mellitus tipo 2 insulinizados ou não.*

MÉTODOS: *Estudo individuado, observacional, descritivo, transversal, comparativo envolvendo 100 pacientes (50 utilizam insulina e 50 não) em um hospital escola. Instrumentos utilizados: Ficha de Identificação e Perfil Socioeconômico; SF-36; Escala Hospitalar de Ansiedade e Depressão; Escala Visual Analógica de Dor; Medida Canadense de Desempenho Ocupacional e Questionário Internacional de Atividade Física.*

RESULTADOS: *Amostra composta, predominantemente, por indivíduos de meia-idade, sexo feminino, casados, ensino fundamental incompleto e aposentados. Há comprometimento de todos os domínios da qualidade de vida, sendo mais intenso nos insulinizados em capacidade funcional, limitação por aspectos físicos, dor, aspectos sociais, limitação por aspectos emocionais e saúde mental (P<0,05). Importante prevalência de sintomas ansiosos ou depressivos nos grupos, principalmente nos em uso de insulina, porém a ocorrência dos transtornos psiquiátricos correspondentes é improvável (P<0,05). Não houve diferença significativa da DNP entre os grupos (P=0,2296). O prejuízo da funcionalidade é semelhante em relação a atividades de autocuidado (P=0,4494) e produtividade (P=0,5759) nos dois grupos, havendo maior deterioração do lazer em usuários de insulina (P=0,0091). A maioria pratica atividade física, tendo a caminhada a maior adesão, sem diferença significativa ao comparar os grupos (P>0,05), repetindo-se nas demais modalidades.*

CONCLUSÃO: *Pacientes insulinizados apresentaram maior prejuízo da capacidade funcional e na socialização, assim como referem maiores dores neuropáticas e sintomas ansiosos e depressivos.*

PALAVRAS-CHAVE: *Diabetes mellitus tipo 2. Insulina. Qualidade de vida. Saúde mental. terapia ocupacional. Exercício.*

REFERENCES

1. Ledón LL. Impacto psicosocial de la diabetes mellitus, experiencias, significados y respuestas a la enfermedad. Rev Cubana Endocrinol. 2012;23(1):76-97.
2. Dalzochio T, Bonho L, Feksa LR, Berlese D. Relationship between depression and diabetes mellitus. Rev Ciênc Méd PUCCAMP. 2014;23(2):91-9.
3. Internatiomal Diabetes Federation. IDF Diabetes atlas. 7nd ed. Bruxelas: Internatiomal Diabetes Federation; 2015.
4. World Health Organization - WHO. Diabetes Programme. Geneva: WHO; 2017. [cited 2017 Aug 5]. Available from: http://www.who.int/diabetes/en/.
5. Higuita-Gutierrez LF, Vargas-Alzate CA, Cardona-Arias, JA. Impacto de la diabetes, el sobrepeso y la obesidad en la calidad de vida relacionada con la salud del adolescente: metanálisis. Rev Chil Nutr. 2015;42(4):383-91.
6. Winkelmann ER, Fontela PC. Condições de saúde de pacientes com diabetes mellitus tipo 2 cadastrados na estratégia saúde da família, em Ijuí, Rio Grande do Sul, 2010-2013. Epidemiol Serv Saúde. 2014;23(4):665-74.
7. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Estratégias para o cuidado da pessoa com doença crônica: diabetes mellitus. Brasília: Ministério da Saúde; 2013.
8. Maia MA, Reis IA, Torres HC. Relationship between the users' contact time in educational programs on diabetes mellitus and self-care skills and knowledge. Rev Esc Enferm USP. 2016;50(1):59-65.
9. Galvin EA, Navarro F, Greatti VR. A importância da prática do exercício físico para portadores de diabetes mellitus: uma revisão crítica. Rev Salusvita. 2014;33(2):209-22.
10. Sociedade Brasileira de Diabetes. Diretrizes da Sociedade Brasileira de Diabetes 2014-2015. Medicamentos orais no tratamento do diabetes mellitus: como selecioná-los de acordo com as características clínicas dos pacientes. São Paulo: Sociedade Brasileira de Diabetes, 2015.
11. Santos SA, Rocha PB, Viana LC. Perfil metabólico de pacientes acometidos por diabetes mellitus tipo II: uma construção educativa. Cad Grad Ciênc Hum Soc Unit. 2015;2(3):65-80.
12. Wexler DJ, Grant RW, Witternberg E, Bosch JL, Cagliero E, Delahany L, et al. Correlates of health-related quality of life in type 2 diabetes. Diabetologia. 2006;49(7):1489-97.
13. Bahety P, Agarwal G, Khandelwal D, Dutta D, Kalra S, Taparia P, et al. Occurrence and predictors of depression and poor quality of life among patients with type-2 diabetes: a Northern India perspective. Indian J Endocrinol Metab. 2017;21(4):564-9.
14. Elissen AMJ, Hertrojis DFL, Schaper NC, Bosma H, Dagnelie PC, Henry RM, et al. Differences in biopsychosocial profiles of diabetes patients by level of glycaemic control and health-related quality of life: the Maastricht study. PLoS One. 2017;12(7):e0182053.
15. Mast R, Rauh SP, Groeneveld L, Koopman AD, Beulens JW, Jansen AP, et al. The use of antidepressants, anxiolytics, and hypnotics in people with type 2 diabetes and patterns associated with use: the Hoorn Diabetes Care System Cohort. Biomed Res Int. 2017;2017:5134602.
16. Lunghi C, Moisan J, Grégoire JP, Guénette L. The association between depression and medication nonpersistence in new users of antidiabetic drugs. Value Health. 2017;20(6):728-35.
17. Grote CW, Wright DE. A role for insulin in diabetic neuropathy. Front Neurosci. 2016;10:581.
18. Callaghan BC, Price RS, Feldman EL. Distal symmetric polyneuropathy: a review. JAMA. 2015;314(20):2172-81.
19. Cortez J, Reis C, Cardoso Y, Onofre A, Piovezan AP. Prevalence of neuropathic pain and associated factors in diabetes mellitus type 2 patients seen in outpatient setting. Rev Dor. 2014;15(4):256-9.
20. Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A; KORA Study Group. Neuropathic pain in diabetes, prediabetes and normal glucose tolerance: the MONICA/KORA Augsburg Surveys S2 and S3. Pain Med. 2009;10(2):393-400.
21. Mendes Z, Guedes S, Guerreiro JP, Inês M, Sousa A, Miranda A. Autovigilância da doença e qualidade de vida dos doentes diabéticos: estudo observacional em farmácias comunitárias. Rev Port Sau Pub. 2016;34(1):11-9.
22. Ferreira MC, Tozatti J, Fachin SM, Oliveira PP, Santos RF, Silva MER. Redução da mobilidade funcional e da capacidade cognitiva no diabetes melito tipo 2. Arq Bras Endocrinol Metab. 2014;58(9):946-52.
23. Alvarenga PP, Pereira DS, Anjos DMC. Mobilidade funcional e função executiva em idosos diabéticos e não diabéticos. Rev Bras Fisioter. 2010;14(6):491-6.
24. Freitas EF, Moreira OC, Oliveira CEP, Doimo LA, Loch MR. Prevalência de diabetes mellitus e prática de exercício em indivíduos que procuraram atendimento na estratégia saúde da família de Viçosa/MG. Rev Educ Fis. 2015;26(4):549-56.
25. Eid LP, Leopoldino SAD, Ollerm GASAO, Pompero DA, Martins MA, Gueroni LPB. Factors related to self-care activities of patients with type 2 diabetes mellitus. Esc Anna Nery. 2018;22(4): e20180046.

ORIGINAL ARTICLE

Response to direct-acting antiviral agents in chronic hepatitis C patients with end-stage renal disease: a clinical experience

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SUMMARY

OBJECTIVE: *The recent development of direct-acting antiviral agents (DAAs) has dramatically changed the treatment of chronic hepatitis C, and interferon-based regimes have become a poor treatment choice in clinical practice. Today DAAs offer shorter, well-tolerated, highly effective curative therapies. This study aimed to evaluate the effectiveness and safety of DAAs in patients with end-stage renal disease and HCV genotype 1 infection in real clinical practice.*

METHODS: *Thirty-six patients who attended our clinic, were diagnosed with chronic hepatitis C (CHC), undergoing hemodialysis, and fulfilled the criteria of age >18 years, genotype 1 infection, with a detectable HCV RNA level were considered for the study. Patients with GT1a infection received OBV/PTV/r plus DSV plus RBV for 12 weeks; GT1b infected patients received this regimen without RBV for 12 weeks.*

RESULTS: *The study was conducted on 33 patients. The mean age was 52.30 ±13.77 years, and 70 % of them were male. By the fourth week of treatment, HCV RNA levels decreased below 15 IU/ml in all patients. Sustained virologic response (SVR) 12 rate was 100%. Nine patients had side effects during treatment. Of the patients with side effects, 89.9% were in group 1a and 11.1% in group 1b.*

CONCLUSION: *In this study, treatment with OBV/PTV/r and DSV with or without RBV resulted in high rates of sustained virologic response in HCV GT1-infected patients with end-stage renal disease (ESRD). SVR was achieved in all patients with few side effects.*

KEY WORDS: *Hepatitis C, End-Stage Renal Disease, Sustained Virologic Response*

INTRODUCTION

Chronic hepatitis C (CHC) infection is a serious global health problem that affects more than 170 million people worldwide. According to reports, 500,000 people worldwide die because of hepatitis C virus (HCV) related liver disease every year.[1] The national prevalence of HCV should be known in order to allow the national healthcare authorities to prioritize preventive measures and manage the use of treatment to reduce resources. Hepatitis C seroprevalence is about 1% in our country. [2] The prevalence of HCV infection

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in patients undergoing hemodialysis is higher than in the general population. In our country, 8.2% of patients undergoing hemodialysis and 4.8 % of patients undergoing peritoneal dialysis are infected with HCV.[3]

The genotype 1 is the most common and includes 11 subgenotypes, of which 1a and 1b are responsible for the vast majority of infections, according to general worldwide statistics. Genotype 1 is also the most common in our country.[4]

Hemodialysis (HD) is one of the great risk factors for HCV, due to contaminated blood-blood product transfusions or exposure to contaminated HD equipment during treatment in uncontrolled places. Thus, HD patients are in risk group lists by the CDC (Center for disease control and prevention) and are recommended to test for HCV routinely. HCV related morbidity and mortality significantly increased in HD patients, especially in developing countries. It is well known that HCV is closely related to kidney function, approximately 10–16% of the patients with HCV infection develop renal disease.[5] In HCV infected patients with renal dysfunction, complications increase due to liver disease, while chronic HCV infection contributes to deteriorating renal function. The incidence of cirrhosis, hepatocellular carcinoma, liver-related mortality, and progression to end-stage renal disease (ESRD) is significantly higher in HCV-infected patients with chronic kidney disease (CKD).[6] In addition, the survival period is shorter in infected patients than that in noninfected ones.

Previously, interferon/pegylated interferon therapy was used in the hepatitis C treatment. Low renal clearance in CKD patients caused interferon-related side effects and increased toxicity.[7] Ribavirin was administered in this group at low doses because renal dose adjustment is required, and anemia is aggravated with ribavirin and interferon.[8] For these reasons, sustained virologic response (SVR) rates are also low (33-37%).[9]

The recent development of direct-acting antiviral agents (DAAs) has dramatically changed the treatment of CHC, and interferon-based regimes have become a poor treatment choice in clinical practice. Today the new direct-acting antiviral regimens (DAAs) offer shorter, well-tolerated, highly efficacious curative therapies. Rates of SVR approach 95%–100% for the treatment of HCV genotype 1 infection. Also, ombitasvir (OBV), paritaprevir (PTV), ritonavir (R) and dasabuvir (DSV) are mainly eliminated by the liver; so, with DAAs, not only the oral use has advantages compared to IFN based therapy, which is an invasive method and administered subcutaneously, but also dose adjustment is not necessary.[10]

This study aimed to evaluate the effectiveness and safety of OBV/PTV/R plus DSV with/without ribavirin (RBV) in patients with ESRD and HCV genotype 1infection in real clinical practice.

METHODS

Patient selection

Thirty-six patients, who attended our clinic between August 2016 and May 2017, were diagnosed with CHC (anti HCV positivity > 6 months), undergoing hemodialysis, and fulfilled the criteria of age >18 years, genotype 1 infection, with a detectable HCV RNA level were coinsidered for the study.

Patients with coinfection by hepatitis B virus or human immunodeficiency virus, non-genotype 1 infection, a history of solid organ transplantation, or on peritoneal hemodialysis were excluded.

At baseline, patients were tested for Anti HCV, HBsAg, Anti HBs, Anti HBcIGg, Anti HIV (ELISA, Liaison, Diasorin, Italy), HCV RNA (Roche COBAS TaqMan real-time reverse transcriptase-polymerase chain reaction assay, version 2.0, lower limit of quantification<15 ıu/ml), genotype (Versant HCV Genotype Inno LiPA Assay, version 2.0), hemogram (Beckman Coulter LH780), and biochemical test (AU 5800 Beckman Coulter kinetical UV method).

Informed consent was obtained from all participants included in the study.

Treatment Regimen

Patients with GT1a infection received OBV/PTV/R (25/150/100 mg once daily) plus DSV (250 mg twice daily) plus RBV (200 mg once daily) for 12 weeks; GT1b infected patients received this regimen without RBV for 12 weeks; the treatments they received for their additional diseases were re-adjusted.

During treatment, patients were monitored at 1, 2, 4, 8, and 12 weeks. At each visit, HCV, RNA, ALT, AST, and hemoglobin levels were measured.

STATISTICS

The analysis was performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, New York, ABD). The descriptive statistics were given as a unit number (n), percentage (%), mean ± standard deviation ($\bar{x}$ ± ss), median (IQR). Pearson

Chi-square and Ficher's exact test were used to evaluate the categorical variables. Normal distributions of the quantitative variables were evaluated by Shapiro Wilk, normality test, and Q-Q graphs. Mann-Whitney U analysis was used for the normal distribution of the two groups, and the Independent Sample T-test was used for the normal distribution. Mann-Whitney U analysis was used in variables with no normal distribution. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Thirty-six ESRD patients who were followed with the diagnosis of CHC were included in the study. Of these, three patients were excluded due to death, missed visits, and abandoning treatment. The study was conducted on 33 patients. The mean age was 52.30 ±13.77 years (22-74), and 70 % of them were male. All patients had been on hemodialysis for 7.6 ± 4.5years.

The mean body mass index (BMI) was 23.52 ±3.77. Demographics, disease characteristics, and laboratory values are presented in Table 1. Thirteen patients were infected with genotype 1b, and 15 with genotype 1a. In five patients, the subtype of genotype 1 could not be analyzed, so it was considered as genotype 1a.

Nineteen patients were treatment-naive, 11 patients had received pegylated IFN, three had received a telaprevir-based regimen. Of the 14 treatment-experienced patients, ten (seven received pegylated IFN, and three received a telaprevir-based regimen) had a partial response, and four had a virologic breakthrough.

Before the start of direct-acting antiviral therapies, median HCV RNA was 2048176±5037964 IU/mL (2170- 18400000). Virologic response rates of genotype 1a and 1b groups at baseline and weeks 1, 2, 4, and 12, and SVR 12 are presented in Table 2. By the fourth week of treatment, HCV RNA levels decreased below 15 IU/ ml in all patients. SVR 12 rate was 100%.

TABLE 1. DEMOGRAGHICS AND DISEASE CHARACTERISTICS (MEAN±STANDART DEVIASION)

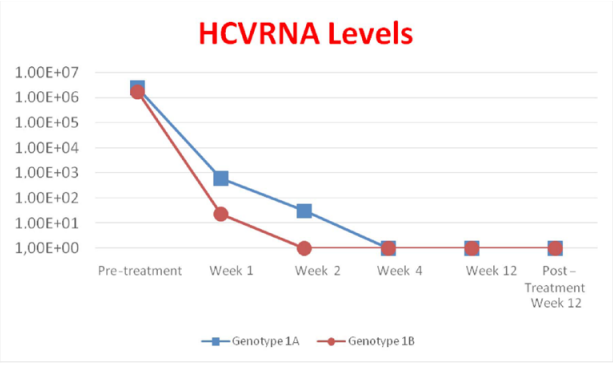
	1A	1B	P-value
Age (year)	51.4±12.1	55.6±13.9	.393
BMI (kg/m2)	24.3±3.2	22.6±3.9	.282
Serum urea (mg/dl)	76.8±36.9	71.5±48.2	.744
Serum creatin (mg/dL)	6.2±2.0	5.9±1.7	.745
Hb (gr/dL)	12.9±1.5	13.8±1.5	.137
WBC (x10^3/u)	7189±2026	7897±2297	.452
ALT (U/L)	33.0±24.1	23.9±13.5	.35
AST (U/L)	26.9±18.2	25.2±12.4	.92
Total Bilirubine (mg/dL)	0.7±0.3	0.6±0.2	.46
PLT (x10^3/uL)	178±65	193±65	.539
AFP (µg/L)	5.3±3.1	2.6±1.5	.019

(BMI; Body Mass Index, Hb; hemoglobin, WBC; White Blood Cell, ALT; Alanine aminotransferase, AST; aspartate aminotransferase, PLT; Platelet, AFP; alpha-fetoprotein)

Nine patients had side effects during treatment. Of these, 89.9% were in group 1A and 11.1% in group 1B. Most side effects were mild, and the most common were fatigue, headache, arthralgia, pruritus, loss of appetite, and stomach pain. Two patients had urticeria, and one had scleral icterus. During the treatment, total bilirubin levels were elevated in five patients for the first two weeks and decreased below normal limits in the first month. (Hyperbilirubinemia was defined as a total bilirubin level of >1.2 mg/dL). During the follow-up, there was no elevation in aminotransferase levels (> 35 U/L). Hemoglobin, leukocyte, and platelet counts at the 12th week after the end of treatment were not significantly different from the baseline values during treatment. There was no difference in the hemoglobin values between treatment regimens with or without RBV.

By the fourth week of treatment, HCV RNA levels decreased below 15 IU/ml in all patients. SVR 12 rate was 100% (Figure 1).

FIGURE 1. VIROLOGIC RESPONSE RATES OF GENOTYPE 1A AND 1B GROUPS.



DISCUSSION

Our study showed that ombitasvir/paritaprevir/ritonavir and dasabuvir are safe and effective in HCV infected CKD patients, and all patients achieved SVR 12.

According to old literature, IFN with or without RBV combination used to be the standard treatment for CHC. Interferon-based therapies in CKD patients have low efficacy, high toxicity, and poor

drug tolerance. Furthermore, side effects such as flu-like symptoms, gastrointestinal, hematological, psychological, and thyroid function test disorders are more common in comparison to patients with normal renal function. Ribavirin also exacerbates anemia. As a result, side effects lead to premature discontinuation of IFN-based therapy protocols.[7,9-11]

Currently, the new DAAs regimens are shorter, interferon-free, well-tolerated, and highly effective therapies. Studies have shown that rates of SVR approach 95%–100% for HCV genotype 1.[12-14]

In the RUBY-I clinical trial, 20 treatment-naive, non-cirrhotic patients with stage 4 or 5 CKD and infected with genotype 1 were evaluated.[15] Thirteen patients with GT1a were administered OBV/PTV/R and DSV, plus RBV (200 mg once daily), and seven patients with GT1b received this regimen without RBV for 12 weeks; the SVR rate reported was 90%. In a study from our country performed by Torun et al. the efficacy and safety of combined therapy of OBV/PTV/R and DSV, with or without ribavirin, were studied in patients who were awaiting a kidney transplant. Patients were divided into two groups according to the genotype; 3/10 were genotype 1a, 7/10 genotype 1b, and the reported SVR 12 rate was 100%.[16] The study published by Etik et al. evaluated 30 CHC patients(18 patients with ESRD and 12 kidney transplant recipients). They reported that SVR 12 was 94% of patients in the ESRD group and 92% in the kidney transplant group.[17]

Sperl et all. evaluated 23 CKD patients infected with HCV GT1 (21 GT1b, 2 GT1a). Six of them had compensated liver cirrhosis.[18] All patients treated with OBV/PTV/R and DSV ± RBV for 12 weeks achieved SVR 12. In our study, the rate of SVR 12 was 100%. Similarly, in the multicenter study carried out by Abad et al., 35 CKD patients infected with genotypes 1 or 4 HCV (seven were cirrhotic) were included, and the SVR rate found was 100%.[19] Although ribavirin was administered at a low dose in hemodialysis patients, there was no decrease in SVR rates.

Miyasaka et al. studied the effectiveness and safety of PTV/OBV/R in 58 (18 were compensated liver cirrhosis) genotype-1 HCV infected patients.[20] The SVR 24 rate reported was 96.6%. Adverse events occurred in 15 patients, but none were severe. They reported that PTV/OBV/R treatment was effective and safe for patients who had chronic hepatitis or compensated hepatic cirrhosis.

We observed that the rate of side effects in genotype 1a was more frequent than in genotype 1b. This also suggests that ribavirin is responsible for side effects. Most side effects were, and the most common were fatigue, headache, arthralgia, pruritus, loss of appetite, and stomach pain. Two patients had urticaria, and one had scleral icterus. Sperl et al. reported that the most common adverse events were nausea, hypotension, diarrhea, and hyperkalemia.[18]

No significant difference was observed in hemoglobin, leukocyte, platelet, and aminotransferase values from baseline and between genotype 1a and 1b. It is known that ribavirin causes dose-dependent anemia; however, anemia was not observed in our study. That may be due to low-dose and short-term use. Torun et

TABLE 2. VIROLOGIC RESPONSE RATES OF GENOTYPE 1A AND 1B GROUPS (MEDIAN - IQR)

	Genotype	Number	Mean	Median	St.Deviation	Minimum	Maximum	p
HCVRNA (IU/ml) Baseline	1A	20	2048176	248000	5037964	2170	18400000	0.828
	1B	13	1716952	149000	2861566	4180	9940000	
HCVRNA(IU/ml) 1st week	1A	10	525		.4 7.5 1479.1	0	4720	0.679
	1B	5	23		.4 0 44.4	0	102	
HCVRNA(IU/ml) 2nd week	1A	12	25	0	79.6	0	277	-
	1B	7	0	-	-	0	0	
HCVRNA (IU/ml) 4th week	1A	17	0	0	-	0	0	-
	1B	12	0	0	-	0	0	
HCVRNA (IU/ml) 12th week	1A	20	0	0	-	0	0	-
	1B	13	0	0	-	0	0	
HCVRNA (IU/ml) post-treatment 12th week	1A	20	0	0	-	0	0	-
	1B	13	0	0	-	0	0	

al. reported that hemoglobin level, white cell blood count, and thrombocyte count were similar to pre-treatment levels and SVR12.[16]

During treatment, total bilirubin levels were elevated in five patients during the first two weeks and decreased below normal limits in the first month.

In a study conducted by Miyasaka et al., 11 of 58 patients had hyperbilirubinemia during treatment. Their focus was that no patient had grade 3 elevated bilirubin levels.[20]

This study has several limitations; The first was its single-center design and the limited number of patients included. The second was that the fibrosis score was not evaluated, and treatment response of other genotypes was lacking; however, the most prevalent genotype in our country is genotype 1. Finally, the post-treatment follow-up period was short to evaluate virologic relapse.

CONCLUSION

In this study, treatment with OBV/PTV/R and DSV, with or without RBV, resulted in high rates of sustained virologic response in HCV GT1-infected patients with ESRD. SVR was achieved in all patients with few side effects.

All HD patients infected with HCV should necessarily be treated if there is no contraindication since they are candidates for renal transplantation, and it is important to prevent HCV complications, such as rejection, proteinuria, diabetes, infection, glomerulopathy associated with HCV, and liver complications in the post-transplant period.

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Authors Contributions

Bengu Tatar: writing, methodology
Şükran Köse: conceptualization, data curation
Nadide Colak Ergun: methodology
Melda Turken: methodology, formal analysis
Yusuf Onlen: supervision
Yusuf Yılmaz: supervision
Sıla Akhan: supervision

RESUMO

O recente desenvolvimento de agentes antivirais de ação direta (DAAs) mudou drasticamente o tratamento da hepatite C crônica, e os regimes livres de interferon tornaram-se pobres escolhas para tratamento na prática clínica. Hoje os DAAs oferecem terapias curativas mais curtas, bem toleradas e altamente eficazes. O objetivo deste estudo foi avaliar a eficácia e segurança dos DAAs em pacientes com doença renal em estágio terminal e infecção pelo genótipo 1 do HCV na prática clínica real.

MÉTODOS: *Trinta e seis pacientes, que se inscreveram em nossa clínica com diagnóstico de hepatite C crônica (CHC), inclusive no programa de hemodiálise, e preencheram os critérios de idade >18 anos, foram considerados para infecção pelo genótipo 1 com nível detectável de RNA do HCV. Os pacientes com infecção por GT1a receberam OBV/PTV/r mais DSV mais RBV por 12 semanas. Os pacientes infectados com GT1b receberam este regime sem RBV por 12 semanas.*

RESULTADOS: *O estudo foi realizado em 33 pacientes. A idade média foi de 52,30±13,77 anos e 70% deles eram do sexo masculino. Na semana 4 do tratamento, os níveis de ARN do VHC diminuíram para menos de 15 UI/ml em todos os pacientes. A taxa de resposta virológica sustentada (RVS) 12 foi de 100%. Nove pacientes apresentaram efeitos colaterais durante o tratamento. Dos pacientes com efeitos colaterais, 89,9% estavam no grupo 1a e 11,1% no grupo 1b.*

CONCLUSÃO: *Neste estudo, o tratamento com OBV/PTV/r e DSV com ou sem RBV resultou em altas taxas de resposta virológica sustentada em pacientes infectados pelo VGC GT1 com doença renal em estágio final (ESRD). A RVS foi alcançada em todos os pacientes com poucos efeitos colaterais.*

PALAVRAS-CHAVE: *Hepatitícos C. Doença renal em estágio terminal. Resposta virológica sustentada.*

REFERENCES

1. Ghany MG, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009;49(4):1335-74.
2. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61(1 Suppl):S45-57.
3. Altuglu I, Soyler I, Ozacar T, Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. Int J Infect Dis. 2008;12(3):239-44.
4. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61(1):77-87.
5. Lens S, Rodriguez-Tajes S, Llovet LP, Maduell F, Londoño MC. Treating hepatitis C in patients with renal failure. Dig Dis. 2017;35(4):339-46.
6. Lee JJ, Lin MY, Chang JS, Hung CC, Chang JM, Chen HC, et al. Hepatitis C virus infection increases risk of developing end-stage renal disease using competing risk analysis. PLoS One. 2014;9(6):e100790.
7. Russo MW, Goldsweig CD, Jacobson IM, Brown RS Jr. Interferon monotherapy for dialysis patients with chronic hepatitis C: an analysis of the literature on efficacy and safety. Am J Gastroenterol. 2003;98(7):1610-5.
8. Bunchorntavakul C, Maneerattanaporn M, Chavalitdhamrong D. Management of patients with hepatitis C infection and renal disease. World J Hepatol. 2015;7(2):213-25.
9. Fabrizi F, Dulai G, Dixit V, Bunnapradist S, Martin P. Meta- analysis: interferon for the treatment of chronic hepatitis C in dialysis patients. Aliment Pharmacol Ther. 2003;18(11-12):1071-81.
10. Gordon CE, Uhlig K, Lau J, Schmid CH, Levey AS, Wong JB. Interferon treatment in hemodialysis patients with chronic hepatitis C virus infection: a systematic review of the literature and meta-analysis of treatment efficacy and harms. Am J Kidney Dis. 2008;51(2):263-77.
11. Alavian SM, Tabatabaei SV. Meta-analysis of factors associated with sustained viral response in patients on hemodialysis treated with standard or pegylated interferon for hepatitis C infection. Iran J Kidney Dis. 2010;4(3):181-94.
12. Pawlotsky JM. New hepatitis C therapies: the toolbox, strategies, and challenges. Gastroenterology. 2014;146(5):1176-92.
13. Ferenci P. Treatment of hepatitis C in difficult-to-treat patients. Nat Rev Gastroenterol Hepatol. 2015;12(5):284-92.
14. Muñoz-Gómez R, Rincón D, Ahumada A, Hernández E, Devesa MJ, Izquierdo S, et al. Theray with ombitasvir/paritaprevir/ritonavir plus dasabuvir is effective and safe for the treatment of genotypes 1 and 4 hepatitis C virus (HCV) infection in patients with severe renal impairment: a multicentre experience. J Viral Hepat. 2017;24(6):464-71.
15. Pockros PJ, Reddy KR, Mantry PS, Cohen E, Bennett M, Sulkowski MS, et al. Efficacy of direct-acting antiviral combination for patients with hepatitis C virus genotype 1 ınfection and severe renal ımpairment or end-stage renal disease. Gastroenterology. 2016;150(7):1590-8.
16. Torun D, Soydas B, Tekkarısmaz N, Ozelsancak R, Mıcozkadıoglu H, Haberal M. Experience with antiviral agents for treatment of hepatitis C virus infection in hemodialysis patients on the kidney wait list. Hemodial Int. 2019;23(3):E78-E82.
17. Özer Etik D, Suna N, Öcal S, Selçuk H, Dağlı Ü, Çolak T, et al. Successful treatment with direct-acting antiviral agents of hepatitis C in patients with end-stage renal disease and kidney transplant recipients. Exp Clin Transplant. 2019;17(1):52-8.
18. Sperl J, Kreidlova M, Merta D, Chmelova K, Senkerikova R, Frankova S. Paritaprevir/ritonavir/ombitasvir plus dasabuvir regimen in the treatment of genotype 1 chronic hepatitis C ınfection in patients with severe renal ımpairment and end-stage renal disease: a real-life cohort. Kidney Blood Press Res. 2018;43(2):594-605.
19. Abad S, Vega A, Hernández E, Mérida E, Sequera P, Albalate M, et al. Universal sustained viral response to the combination of ombitasvir/paritaprevir/ritonavir and dasabuvir with/without ribavirin in patients on hemodialysis ınfected with hepatitis C virus genotypes 1 and 4. Am J Nephrol. 2017;45(3):267-72.
20. Miyasaka A, Yoshida Y, Yoshida T, Murakami A, Abe K, Ohuchi K, et al. The real-world efficacy and safety of ombitasvir/paritaprevir/ ritonavir for hepatitis C genotype 1. Intern Med. 2018;57(19):2807-12.

ORIGINAL ARTICLE

Reducing overcrowding in an emergency department: a pilot study

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SUMMARY

OBJECTIVE: *Exploring the use of forecasting models and simulation tools to estimate demand and reduce the waiting time of patients in Emergency Departments (EDs).*

METHODS: *The analysis was based on data collected in May 2013 in the ED of Recanto das Emas, Federal District, Brasil, which uses a Manchester Triage System. A total of 100 consecutive patients were included: 70 yellow (70%) and 30 green (30%). Flow patterns, observed waiting time, and inter-arrival times of patients were collected. Process maps, demand, and capacity data were used to build a simulation, which was calibrated against the observed flow times. What-if analysis was conducted to reduce waiting times.*

RESULTS: *Green and yellow patient arrival-time patterns were similar, but inter-arrival times were 5 and 38 minutes, respectively. Wait-time was 14 minutes for yellow patients, and 4 hours for green patients. The physician staff comprised four doctors per shift. A simulation predicted that allocating one more doctor per shift would reduce wait-time to 2.5 hours for green patients, with a small impact in yellow patients' wait-time. Maintaining four doctors and allocating one doctor exclusively for green patients would reduce the waiting time to 1.5 hours for green patients and increase it in 15 minutes for yellow patients. The best simulation scenario employed five doctors per shift, with two doctors exclusively for green patients.*

CONCLUSION: *Waiting times can be reduced by balancing the allocation of doctors to green and yellow patients and matching the availability of doctors to forecasted demand patterns. Simulations of EDs' can be used to generate and test solutions to decrease overcrowding.*

KEYWORDS: *Time Management, Emergency Medical Services, Computer Simulation, Health Services Needs and Demand, Patient Satisfaction.*

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INTRODUCTION

The primary concern of (medical) emergency departments (EDs) is the ability to provide timely and effective care.[1-5] However, the operations of EDs are highly complex, and the ability of the staff to provide good care is affected by many factors. Overcrowding is one such factor and a very common problem that EDs face worldwide. It reduces the quality of care, worsens the work environment, increases the time necessary to initiate appropriate patient treatment, and raises the cost of the healthcare system.[1-3,6]

The number of patients entering EDs at a given time is the greatest factor influencing wait-times for an initial consultation, as well as an individual's total time in an ED. The performance of the ED can also be exacerbated by other factors such as the mismanagement of patient flows, lack of access to inpatient beds, demand from patients for specialized care instead of primary care, absence of or reduced access to primary care, and structural failure of the healthcare system to manage population health.[2,6,7]

While overcrowding is a widespread problem, it follows a predictable pattern—across hours, days, weeks, months, and seasons—of patients arriving at EDs.[2] With the capabilities of information technology, it is possible to both understand this pattern and build simulation tools that can assist managers of healthcare systems. These tools can predict demand and wait times, which would help managers plan human resources and alter processes and patient flows to improve the quality of care while respecting budget constraints. Furthermore, these tools can be used to train staff and inform patients, thereby further improving the performance of EDs.[1,2,4,6]

The main purpose of this study was to build a forecasting model and a simulation tool that would predict the demand and waiting time of patients in an ED, assist in human resource planning, process design, guide educational strategies for staff, and improve the decision-making processes of healthcare managers.

METHODS

This study is based on data collected in May 2013 in the ED of Recanto das Emas, Federal District, Brasil, which is a public ED of the Brazilian Single Health System (SUS). The Manchester Triage System (MTS) was used to triage patients. In the MTS, different colors indicate the level of urgency and its associated maximum wait time. The colors and their respective levels of urgency are as follows: red, immediate care by a physician (0 minutes); orange, very urgent (10 minutes); yellow, urgent (60 minutes); green, standard care (2 hours); blue, non-urgent care (4 hours).[8]

The following method was employed in this study.

Step A - Data collection: In this step, the following information was collected

1) Mapping of the general process of patient flows for yellow and green levels (figure 1);

2) Collection of arrival and processing times. This was done over six consecutive days.

3) Availability of physicians. This information was obtained from physicians' work schedules

Step B: Model development and validations

The data collected was used to develop the following:

Flow patterns of patients through the ED for each patient class (MTS color),

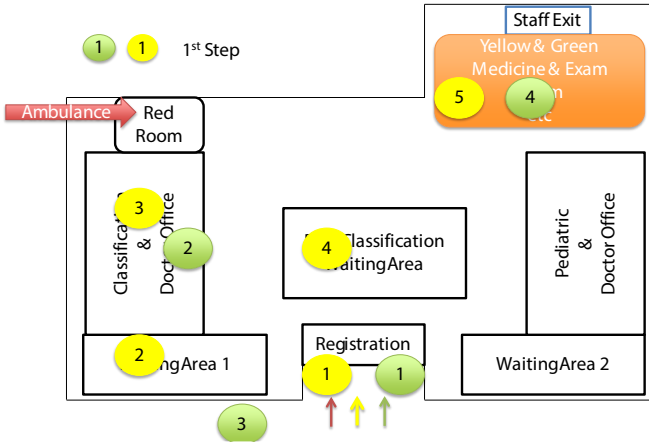
Observed waiting time in the ED (the length of time when the patient is waiting for a physician),

Inter-arrival times (the time-gap between arrivals of two consecutive patients in the ED), and the distribution of the number of arrivals during the period of a day were determined (figure 2).

The process maps, demand, and capacity data were used to build a simulation tool in Simul8.

The simulation output was calibrated against the observed flow times, and what-if analyses were conducted to reduce waiting times

FIGURE 1. MAPPING OF THE GENERAL PROCESS OF PATIENT FLOWS FOR YELLOW AND GREEN LEVELS IN THE EMERGENCY DEPARTMENT OF RECANTO DAS EMAS, FEDERAL DISTRICT, BRASIL.

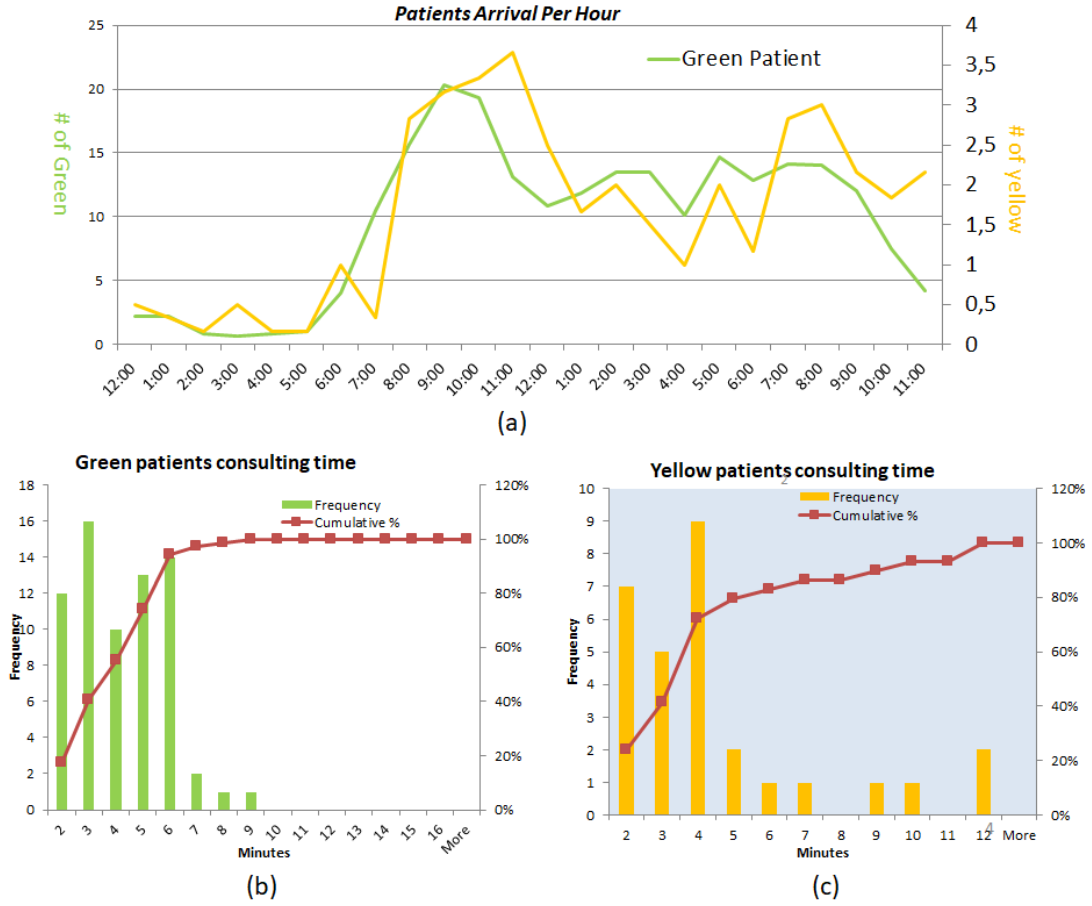


RESULTS

The process maps and the simulation tool yielded the following findings. (figure 1):

1. **Entry to the System:** After a patient enters the system, they register at the front desk, where administrators and a nurse are present. That nurse's function is to identify emergency care patients that should be taken directly to the "red room" where a "red doctor" works as a dedicated resource. Other walk-in patients wait an average of 20 minutes before being further triaged by a nurse practitioner. The nurse assesses the MTS level and routes the patient.
2. **Initial Triage and Perceived Lack of Progress:** Of approximately 300 patients per day that enter the UPA system, approximately 210 patients are green, approximately 35 are yellow, and the remainder are blue, orange, or red patients. Green patients get classified and, instead of moving forward to the Post-Classification Waiting Area, they return to the Waiting Area #1 or #2. The area allocated to green patients is small, leading to crowding. The green patients feel they are not moving through the system, which leads them to believe they are not important.
3. **Consultations with a Physician and Delays:** For each 8-hour shift, there are six doctors on call. One is dedicated to pediatrics. One is dedicated to the red-level. One is dedicated to yellow patients in the "yellow room" (patients with more urgent needs, staying in the system, or in need of a transfer to the hospital). The three remaining doctors are stationed in the Classification Doctors Office area. These three doctors diagnose patients after classification and control the flow subsequently in the system. Due to the priority label given to

FIGURE 2. DISTRIBUTION OF THE NUMBER OF ARRIVALS DURING THE DAY AND TIME SPENT DURING A CONSULTATION FOR YELLOW AND GREEN LEVELS IN THE EMERGENCY DEPARTMENT OF RECANTO DAS EMAS, FEDERAL DISTRICT, BRASIL.



(a) Distribution of the number of arrivals during the day and the time spent during a consultation for yellow and green levels, (b) time spent during a consultation for green level, and (c) time spent during a consultation for yellow level.

yellow patients, green patients may, on average, spend to up to two hours waiting to see a doctor, and four hours in the system, while yellow patients typically get seen within 20 minutes and move through the system at a quicker rate.

To address the problems identified above, the following solutions were proposed.

1. **Initial Triage and Lack of Progress:** One possible solution to address green patients' dissatisfaction is to create a dual-door system out of the nurse's Classification Office. The entry and exit doors would be different, as would in the waiting area. This could create a sense of progress.
2. **Consultation with a Physician and Delays:** The number of arrivals per hour was determined across all six days for green and yellow patients separately. The data shows that the arrival patterns according to the time of the day, both for yellow and green levels. The study included 100 consecutive patients: 70 yellow patients (70.0%) and 30 green patients (30%). The data shows that green and yellow patient hourly arrival patterns are similar across all days. For yellow patients, 9:00-11:00 a.m. was the peak arrival time, and 8:00-10:00 a.m. was the peak arrival time for green patients (figure 2).

The mean inter-arrival time was 38 minutes for yellow patients, and 5 minutes for green patients. In the period of the study, the ED had a physician staff of four doctors per shift, at all times. The mean wait-time was 14 minutes for yellow patients, and 4 hours for green patients (table 1). The time spent during a consultation with a doctor was 4.3 minutes for yellow patients and 4.1 minutes for green patients (figure 2).

A simulation model was built to conduct what-if analysis. The alternatives considered were (a) adding more physicians and (b) changing priorities.

(a) *Adding an extra physician*: The simulation model predicts that allocating one more doctor per shift (i.e., five doctors per shift) would be associated with a reduction in the average wait-time of 2.5 hours for green patients, as well as a small reduction in the average wait-time for yellow patients.

(b) *Retaining the same 4 physicians but dedicating one to the green* area: maintaining four doctors and allocating one exclusively for green patients would be associated with a reduction in the average waiting time of 1.5 hours for green patients and an increase in the average waiting time of 15 minutes for yellow patients,

(c) *Adding an extra physician and dedicating two to the green* area: this scenario employed five doctors per shift with two doctors allocated exclusively for green patients. In this scenario, the average waiting time was reduced to 2.8 hours for green patients and increased by 7 minutes for yellow patients (table 1).

TABLE 1. SIMULATION MODEL USED TO CONDUCT WHAT-IF ANALYSIS IN THE EMERGENCY DEPARTMENT OF RECANTO DAS EMAS, FEDERAL DISTRICT, BRASIL.

4 Physicians	No Dedicated "Green" Doctor (Status Quo)	1 Dedicated "Green" Doctor
	Yellow waiting time: 14 min	Yellow waiting time: 29 min
	Green waiting time: 4 hours	Green waiting time: 2.5 hours
5 Physicians	No Dedicated "Green" Doctor	2 Dedicated "Green" Doctors
	Yellow waiting time: 15 min	Yellow waiting time: 21 min
	Green waiting time: 1.5 hours	Green waiting time: 1.2 hours

Assumption:
- 80/20 split: 80% of green patients go to dedicated green doctors
- Inter-arrival time: "green" every 5 minutes vs. "yellow" every 38 minutes
- Average doctor consultation time: 10 minutes for both "green" & "yellow" patients

DISCUSSION

The performance of healthcare services is under constant scrutiny. A dramatic increase in EDs visit rates—a global phenomenon—has led to overcrowding, long waiting times, and delays in critical treatments. This has become a major problem for health care managers, as a patient's wait-time is one of the most important factors in health care management and a great determinant of patients' satisfaction.[2,3-6,10,11] Because of this, the need for improvement in EDs with regards to overcrowding and patient satisfaction is broadly accepted.[9] Data-driven analysis has been used successfully to improve patient flow in EDs. The resulting improvements consist of optimal provisioning of available resources.[12] Healthcare managers can adjust staffing and flow rules based on the observed patterns and experience substantial improvement in the ED's performance.[1,2,4,6,9,12]

In this study, we used sample data to model the arrival rates, flow patterns, and processing times. This was used to set up a simulation model that can predict the impact of different staffing and flow control

rules on the wait-time in the ED for different patient classes. This enables healthcare managers to generate and evaluate solutions to improve the current overcrowding situation under different scenarios.

In the present study, although yellow patients were being seen within the wait-time recommended by the MTS, the average wait-time for green patients was twice as long as recommended by the MTS. A simulation tool was used to evaluate solutions that would reduce the waiting time for green patients. The most effective solution, which ensured no increase in the waiting time for yellow patients and brought the green waiting time down to conformance levels, employed five doctors per shift with two of those doctors allocated exclusively for green patients.[8] This analysis brings into sharp focus the trade-offs between the cost of additional resources and improved patient experience.

If increasing the staff to five doctors is not possible, the simulation tool suggests that allocating a single doctor exclusively for green patients would be associated with a reduction in the average wait-time of 1.5 hours for green patients, while maintaining the wait-time for yellows patients under 60 minutes—the maximum waiting time suggested by MTS.[8] The analysis also shows that priority rules can also alter the performance. In other words, even without adding resources, by managing the flows carefully, it is possible to yield significant improvements. This illustrates the importance of using data to develop optimal flow management rules, especially in budget-constrained environments where adding resources is not feasible.

Ideally, the human resources of an ED need to match the workload as dictated by patients' arrival-time patterns.[1,2,6,13] . A mismatch between the arrival rates of patients and the number of employees on staff can be a major contributing factor to overcrowding and prolonged wait-times in EDs.[1,6,13] . In this study, a pattern of patients' arrival time was observed with peaks in the morning and trough at night. This pattern is similar to that of other studies.[6,14-16] The patients' arrival pattern suggests that the relocation of one doctor from the night-shift to the day-shift could also improve the wait-time without requiring additional human resources.

One of the main limitations of the study is its small sample size and short duration. Indeed, it should be highlighted that having a simulation tool and implementing it to alter clinical practice are two different things. In this case, this tool has only been tested in simulation scenarios and, consequently, the researchers will need to determine if it has an impact on clinical care. The next step is to apply the simulation tool as an automated system to predict wait-times and to manage patient flow on a real-time basis in an ED. In this case, the patients would be able to see the ED waiting times online. This information can help patients arrive at the ED during less-crowded hours.[17] Reduced waiting times not only increase patient satisfaction but will also enhance patient safety. Previous studies have shown an association between ED overcrowding and an increase in patients departing from the EDs without being seen by a physician, as well as adverse events such as medication errors and infection transmissions.[18-22]

CONCLUSION

Data-driven analysis can be used to assist healthcare managers in providing solutions to improve the current overcrowding situation. Data can be used to carefully balance resource availability with patient arrivals. This may entail changing staffing levels and rules used to manage flows. For example, the most effective of the different scenarios to reduce the extremely high wait-times for green patients —without increasing the wait-times for yellow patients—was to employ five doctors per shift, with two of those doctors allocated exclusively for green patients. Also, shifting some capacity from relative slow evening periods to busier mornings can sharply reduce waiting times. Overcrowding can also be reduced by influencing arrival patterns. For example, a real-time tool that predicts waiting times can be used to inform patients. This, in turn, can cause the peak load to dissipate.

We observed some fragilities of the system that impact the patients' perception of their value. This example illustrates that patient satisfaction can also depend on subjective factors. As discussed, one possible solution to improve patient experience is to create a dual-door system at the nurse's Classification Office. This would help patients feel that they are moving forward in the system, and therefore are important.

This work shows that data analysis can facilitate optimal staffing and management of flows. In resource-constrained environments where adding resources is not easy, optimal usage of staff is crucial. Balancing the scheduling of available doctors to hourly demand patterns and allocating the right number of doctors to green and yellow patients can diminish

waiting times and improve patient safety, even without increasing staffing levels. Hence, the analysis also illustrates the importance of leveraging data and creating digitally ready emergency departments, especially in resource-constrained environments.

Acknowledgments

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Conflicts of interest: None.

RESUMO

OBJETIVO: *Avaliar a frequência e a gravidade de erros em prescrições envolvendo medicamentos potencialmente perigosos (heparina e cloreto de potássio concentrado injetável) antes e após a introdução de um sistema de prescrição eletrônica.*

MÉTODOS: *Trata-se de estudo retrospectivo que comparou erros em prescrições manuais e pré-digitadas de 2007 (Fase 1) com prescrições eletrônicas de 2014 (Fase 2) (total = 1.028 prescrições), em dois hospitais de alta complexidade de Belo Horizonte.*

RESULTADOS: *Foi observado no hospital 1 aumento de 25% dos erros depois da intervenção (p<0,001), e no hospital 2 foi verificada redução de 85% (p<0,001). Para o cloreto de potássio, a frequência de erros permaneceu a mesma no hospital 1 (p>0,05), independentemente da fase e, no hospital 2, ocorreu redução significativa na fase 2 (p<0,001). Foi identificada redução da gravidade dos erros com a heparina (p<0,001), mas não houve alteração na gravidade dos erros com cloreto de potássio (p>0,05).*

CONCLUSÕES: *A frequência e a gravidade dos erros de medicação após a introdução de prescrição eletrônica foram impactadas de forma diferente nos dois hospitais, demonstrando necessidade de observação criteriosa quando o sistema de prescrição é modificado. Durante e após a implantação dessa tecnologia, deve existir controle dos novos erros potenciais introduzidos e suas causas para a adoção de medidas de prevenção desses eventos.*

PALAVRAS-CHAVE: *Segurança do paciente. Prescrição eletrônica. Erros de medicação. Prescrições de medicamentos.*

REFERENCES

1. Anderson D, Pimentel L, Golden B, Wasil E, Hirshon JM. Drivers of ED efficiency: a statistical and cluster analysis of volume, staffing, and operations. Am J Emerg Med. 2016; 34(2):155-61.
2. Crade J, Noon C. The definitive guide to emergency department operational improvement: employing lean principles with current ED best practices to create the "no wait" department. 1. ed. New York: Productivity Press, 2011.
3. Pines JM, Hilton JA, Weber EJ, Alkemade AJ, Al Shabanah H, Anderson PD, et. al. International perspectives on emergency department crowding. Acad Emerg Med. 2011; 18(12):1358–70.
4. Ajami S, Ketabi S, Yarmohammadian MH, Bagherian H. Wait Time in Emergency Department (ED) Processes. Med Arh. 2012; 66(1):53-7.
5. Choyce MQ, Maitra AK. Satisfaction with the Accident and Emergency Department, a Postal Survey of General Pracitioners' Views. J Accid Emerg Med. 1996; 13(4):280-2.
6. Tiwari Y, Goel S, Singh A. Arrival time pattern and waiting time distribution of patients in the emergency outpatient department of a tertiary level health care institution of North India. J Emerg Trauma Shock. 2014; 7(3):160-5.
7. Harris A, Sharma A. Access block and overcrowding in emergency departments: an empirical analysis. Emerg Med J. 2010; 27(7):508-11.
8. Mackway-Jones K, Marsden J, Windle J (Eds.). Emergency Triage: Manchester Triage Group. 3. ed. Massachussets: Wiley Blackwell, 2013.
9. Holden RJ. Lean Thinking in emergency departments: a critical review. Ann Emerg Med. 2011; 57(3):265-78.
10. Higginson I. Emergency department crowding. Emerg Med J. 2012; 29(6):437-43.
11. Cooke M. Emergency medicine. Whole system is responsible for solving overcrowding of departments. BMJ. 2002; 325(7360):389.
12. Lovett PB, Illg ML, Sweeney BE. A Successful Model for a Comprehensive Patient Flow Management Center at an Academic Health System. Am J Med Qual. 2016; 31(3):246-55.
13. Singer AJ, Thode HC Jr, Viccellio P, Pines JM. The Association Between Length of Emergency Department Boarding and Mortality.Acad Emerg Med. 2011; 18(12):1324-9.
14. Goel S, Singh A. Will plague continue to haunt hilly states of India? The Internet J Health. 2007; 8(1):18–23.
15. Chan L, Reilly KM, Salluzzo RF. Variables that affect patient through put times in an academic emergency department. Am J Med Qual. 1997; 12(4):183–6.
16. Ay D, Akkas M, Sivri B. Patient population and factors determining length of stay in adult ED of a Turkish University Medical Center. Am J Emerg Med. 2010; 28(3):325–30.
17. [No authors listed]. Patients in Northern Ireland can see waiting times online. Emerg Nurse. 2015; 23(7):6.
18. Kulstad EB, Sikka R, Sweis RT, Kelley KM, Rzechula KH. ED overcrowding is associated with an increased frequency of medication errors. Am J Emerg Med. 2010; 28(3):304–9
19. Goel S, Gupta AK, Singh A, Lenka SR. Preparations and limitations for prevention of severe acute respiratory syndrome in a tertiary care centre of India. J Hosp Infect. 2007; 66(2):142–7.
20. Monzon J, Friedman SM, Clarke C, Arenovich T. Patients who leave the emergency department without being seen by a physician: a control-matched study. CJEM. 2005; 7(2):107–13.
21. Weiss SJ, Ernst AA, Derlet R, King R, Bair A, Nick TG. Relationship between the national ED overcrowding scale and the number of patients who leave without being seen in an academic ED. Am J Emerg Med. 2005; 23(3):288–94.

ORIGINAL ARTICLE

Mortality in motorcycle accidents in Alagoas (2001-2015): temporal and spatial modeling before and after the "lei seca"

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SUMMARY

OBJECTIVE: *To analyze the epidemiological profile and the Spatio-temporal distribution of mortality in motorcycle accidents in Alagoas before (2001-2007) and after the "Lei seca" (2008-2015).*

METHODS: *This is a mixed ecologic study. All deaths in the state with the codes V20-V29 (ICD-10) as the basic cause were included in the study. Sociodemographic variables and mortality rates per sex were analyzed. For the temporal analysis, the inflection point regression model was used. For spatial analysis, the rates were smoothed by the Local Empirical Bayesian Model and, subsequently, the Global and Local Moran statistic was used to identify the spatial clusters of risk.*

RESULTS: *There were 1458 deaths caused by motorcycle accidents in the period studied; the following characteristics about the victims stand out: male (91.29%), economically active age (82.93%), and brown race (78.12%). In the male population, there was a growth trend between 2001 and 2007 (19.0%, p<0.001), and a decline from 2008 (-11.2%, p<0.001). Spatial modeling showed that the areas with the highest risk of mortality are located in the agreste and sertão of the state (p = 0.01).*

CONCLUSION: *Mortality in motorcycle accidents is an important public health problem in Alagoas, with an emphasis on male mortality and geographic concentration within the state.*

KEYWORDS: *Accidents, Traffic. Ecological Studies. Mortality Registries.*

INTRODUCTION

Over the past decades, traffic-related deaths and injuries have become an important global public health problem. In 2016 alone, 1.35 million people died worldwide due to traffic accidents, and the mortality rate is three times higher in low-income countries[1].

In that same year, 37,345 deaths were reported due to traffic accidents in Brasil, with a rate of 18.1/100,000 inhabitants. Of these, 32% involved two- or three-wheel vehicles[1]. It is estimated that the risk of a fatal outcome in a motorcycle accident is 20 times higher than in car accidents[2].

In 2008, in an attempt to reduce the number of traffic accidents, Law no. 11,705, popularly known as "*Lei Seca*", was enacted. Assessing the impact of

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the legislation on the temporal and spatial pattern of traffic mortality is imperative for public health and safety[3,4], in view of the magnitude of the problem and the need to understand the phenomenon in different geographical regions of the country and identify priority areas for intervention[4].

Considering alcohol as the main risk factor for traffic mortality and the motorcyclist population as the most vulnerable, this study aimed to analyze the epidemiological profile and the Spatio-temporal distribution of mortality in motorcycle accidents in Alagoas before (2001-2007) and after the *Lei Seca* (2008-2015).

METHODS

This is a mixed ecologic study. This work was carried out in Alagoas, considering the period 2001-2015. The temporal series was subdivided into two, and the temporal milestone adopted was the year 2008, when the law entered into force. The state comprises 102 municipalities and a population of over 3 million inhabitants; it is the most densely populated area in the Northeast Region (112.13 inhabitants/km²)[5].

We included all deaths due to transport accidents involving motorcycles and tricycles (driver/passenger), considering ICD-10, codes V20-V29: V20 - Motorcycle rider injured in collision with pedestrian or animal; V21 - Motorcycle rider injured in collision with pedal cycle; V22 - Motorcycle rider injured in collision with two- or three-wheeled motor vehicle; V23 - Motorcycle rider injured in collision with car, pick-up truck or van; V24 - Motorcycle rider injured in collision with heavy transport vehicle or bus; V25 - Motorcycle rider injured in collision with railway train or railway vehicle; V26 - Motorcycle rider injured in collision with other nonmotor vehicle; V27 - Motorcycle rider injured in collision with fixed or stationary object; V28 - Motorcycle rider injured in noncollision transport accident V29 - Motorcycle rider injured in other and unspecified transport accidents - NE. These data were obtained from the Mortality Information System (SIM) from the Datasus platform (http://datasus.saude.gov.br/).

Sociodemographic variables were collected in order to characterize the population studied: sex, age, race/color, formal education, marital status, place of death, and the ICD category. These variables were subjected to descriptive analysis. Then, the mortality rates in the general population and according to sex were calculated.

For the temporal analysis, we used the joinpoint regression model[6]. The trends were classified as stationary, ascending, or descending, and the Annual Percentage change (APC) and the Average Annual Percent Change (AAPC) were calculated. We adopted a significance of 5% and a confidence interval of 95%.

Then, spatial analysis was conducted. Initially, we applied the local empirical Bayes model to the mortality rates to give greater stability to the data. After that, the spatial dependence of the indicators was evaluated using the statistic of the global Moran's statistic and the pseudo-significance test. Once global spatial dependency was observed, we applied the Local Index of Spatial Association - Lisa. From the Lisa, each municipality was placed in one quadrant of the Moran scatter plot: Q1 - High/high (positive values and positive means), Q2 - low/low (negative values and negative means), Q3 - high/low values (positive values and negative means), and Q4 - low/High (negative values and positive means). Based on the results obtained, the thematic maps were built to identify spatial clusters of higher risk of deaths[7]. For the analyses, we used the Terra View 4.2.2, QGis 2.14.11, and Joinpoint Regression Program 4.5.0.1 software.

Since the study used secondary data from information systems of public domain, it was waived approval by the Research Ethics Committee.

RESULTS

Between 2001 and 2015, 1,458 deaths were recorded due to motorcycle accidents in the state of Alagoas, 1,331 (91.29%) of the victims were males, 39.09% (n=570) aged between 20 and 29 years, and 78.12% (n=1139) of mixed race. There was a predominance of deaths in hospital units (50.34%; n=734), followed by on public roads (44.58%; n=650) (Table 1); 50.14% (n=731) of deaths resulted from transport accidents without collision (Table 1).

The mortality rate in the period was 3.06 deaths/100 thousand inhabitants (5.69/100,000 for men, and 0.52/100,000 for women). In the period prior to the *Lei Seca* (2001-2007), there was a statistically significant growth of overall mortality (APC 18.9%; $p<0.001$) and among males (APC 19.0%; $p<0.001$). In the period after the law went into force (2008-2015), there was a trend reversal, with a decline of -11.3% ($p<0.001$) in the overall mortality and -11.2% ($p<0.001$) among men. Among women, the behavior was constant in both periods (Table 2).

The spatial distribution showed expansion of

TABLE 1. SOCIODEMOGRAPHIC CHARACTERISTICS OF DEATHS FROM MOTORCYCLE TRAFFIC ACCIDENTS. ALAGOAS, BRASIL, 2001-2015 (N=1,458).

Variable	Male 1,331 (91.29%)		Female 127 (8.71%)		Total 1,458 (100.0%)	
	n	%	n	%	n	%
Age range						
< 10 years	4	0.31	1	0.79	5	0.35
10 a 14	14	1.05	4	3.15	18	1.23
15 a 19	140	10.52	20	15.75	160	10.97
20 a 29	523	39.29	47	37.01	570	39.09
30 a 39	319	23.97	28	22.05	347	23.80
40 a 49	196	14.73	11	8.66	207	14.20
50 a 59	78	5.86	7	5.51	85	5.84
60 or more	57	4.29	9	7.09	66	4.52
Race/color						
White	96	7.21	17	13.39	113	7.75
Black	16	1.20	2	1.57	18	1.23
Yellow	2	0.15	1	0.79	3	0.21
Brown	1,050	78.89	89	70.08	1,139	78.12
Indigenous	1	0.08	0	0.00	1	0.07
Ignored	166	12.47	18	14.17	184	12.62
Years of formal education						
None	19	1.43	2	1.57	21	1.44
1 to 3 years	48	3.61	2	1.57	50	3.43
4 to 7 years	182	13.67	16	12.60	198	13.58
8 to 11 years	70	5.26	9	7.09	79	5.42
12 or more	7	0.53	0	0.00	7	0.48
Ignored	1,005	75.51	98	77.17	1103	75.65
Site						
Hospital	667	50.11	67	52.76	734	50.34
Other health establishments	4	0.30	0	0.00	4	0.27
Home	15	1.13	1	0.79	16	1.10
Public Road	594	44.63	56	44.09	650	44.58
Others	51	3.83	3	2.36	54	3.71
ICD-10 Category						
V20	10	0.75	0	0.00	10	0.69
V21'	-	-	-	-	-	-
V22	18	1.35	1	0.79	19	1.30
V23	82	6.16	14	11.02	96	6.58
V24	30	2.25	4	3.15	34	2.33
V25'	-	-	-	-	-	-
V26	11	0.83	0	0.00	11	0.75
V27	15	1.13	1	0.79	16	1.10
V28	674	50.64	57	44.88	731	50.14
V29	491	36.89	50	39.37	541	37.11

Legend: V20 - Motorcycle rider injured in collision with pedestrian or animal; V21 - Motorcycle rider injured in collision with pedal cycle; V22 - Motorcycle rider injured in collision with two- or three-wheeled motor vehicle; V23 - Motorcycle rider injured in collision with car, pick-up truck or van; V24 - Motorcycle rider injured in collision with heavy transport vehicle or bus; V25 - Motorcycle rider injured in collision with railway train or railway vehicle; V26 - Motorcycle rider injured in collision with other nonmotor vehicle; V27 - Motorcycle rider injured in collision with fixed or stationary object; V28 - Motorcycle rider injured in noncollision transport accident V29 - Motorcycle rider injured in other and unspecified transport accidents - NE. * No records of death in these categories.

TABLE 2. TEMPORAL EVOLUTION AND MORTALITY RATE TREND IN MOTORCYCLE TRAFFIC ACCIDENTS (PER 100,000 INHABITANTS), OVERALL AND ACCORDING TO SEX. ALAGOAS, BRASIL, 2001-2015 (N=1,458).

(a) Mortality rate in motorcycle accidents						
Year	Overall mortality		Male mortality		Female mortality	
	No. deaths	Rate/100,000	No. deaths	Rate/100,000	No. deaths	Rate/100,000
2001	33	1.12	29	2.00	4	0.27
2002	60	2.02	59	4.02	1	0.07
2003	70	2.32	64	4.31	6	0.39
2004	84	2.75	71	4.73	13	0.84
2005	103	3.34	94	6.19	9	0.57
2006	111	3.56	104	6.79	7	0.44
2007	129	4.10	118	7.63	11	0.69
2008	116	3.65	107	6.86	9	0.56
2009	118	3.68	109	6.94	9	0.55
2010	124	3.84	115	7.27	9	0.55
2011	118	3.62	108	6.78	10	0.60
2012	129	3.93	116	7.24	13	0.77
2013	142	4.30	126	7.83	16	0.95
2014	58	1.75	53	3.28	5	0.29
2015	63	1.89	58	3.57	5	0.29
2001-2015	1,458	3.06	1,331	5.69	127	0.52
(b) Joinpoint Regression Model						
Period	APC (CI 95%) p-value		APC (CI 95%) p-value		APC (CI 95%) p-value	
2001-2007[a]	18.9 (12.0 to 26.3) p<0.001*		19.0 (9.9 to 28.9) p<0.001*		4.9 (-3.3 to 13.9) p=0.2	
2008-2015[b]	-11.3 (-18.5 to -3.6) p<0.001*		-11.2 (-20.5 to -0.7) p<0.001*		4.9 (-3.3 to 13.9) p=0.2	

Legend: * Statistical significance; APC: Annual Percent Change; AAPC: Average Annual Percent Change; CI 95%: Confidence interval of 95%; a Period before the Lei Seca went into force in Brasil; b Period the Lei Seca went into force in Brasil.

mortality over the time series, as well as a spatial dependency (p=0.01). Before the *Lei Seca*, the greatest overall rates corrected were found in Coité do Noia (7.8/100,000), Craíbas (7.22/100,000), and Arapiraca (6.93/100,000). These municipalities also occupied the top three positions in the ranking of male mortality (14.32/100,000, 13.8/100,000, and 6.93/100,000, respectively). In the female population, the municipalities of Coité do Noia (1.44/100,000), Feira Grande (1.56/100,000), and São Sebastião (1.51/100,000) stood out (Figure 1).

In the post-enforcement period, the municipalities of Taquarana (7.43/100,000), Arapiraca (6.91/100,000), and Cacimbinhas (6.86/100,000) stood out for the general mortality; Taquarana (14.13/100,000), Cacimbinhas (13.59/100,000), and Porto Real do Colégio (12.95/100,000) for male mortality; and Pão de Açúcar (2.11/100,000), São José da Tapera (2.11/100,000), and Monteirópolis (1,82/100,000) for female mortality (Figure 1).

The expansion of mortality was also observed in the Moran Map. For the overall mortality, the number of municipalities located in quadrant 1 (Q1) of the Moran scatter plot went from 20 (19.60%), before the *Lei Seca,* to 33 (32.35%) in the period after it went into force. In the male population, the growth was similar, from 21 (20.58%) to 32 (31.37%). In both cases, the municipalities were concentrated in the central region of the state (agreste of Alagoas and transition with the sertão). In the female population, the same number of municipalities was observed (n=19; 18.62%) in both periods; however, there was a change in the spatial pattern, with an expansion of mortality to the sertão of the state.

DISCUSSION

The male mortality rate 10.9 times higher than that of females observed in Alagoas corroborates the national and international literature[8 1]. Of the 38,000

accidents with fatal victims recorded in Brasil in 2015, 82% of deaths were of men[1]. Various aspects can justify this context, highlighting, initially, the higher consumption of motorcycles by the male population[9]. Approximately 85% of purchases of such vehicles in the country are by men, and 83% of purchasers are less than 40 years old[10].

In association with the consumption profile, the male population has a higher prevalence of risk behaviors, such as the consumption of alcohol[11], excess speeding[8], no use of personal protective equipment[11], driving without a license[12], and lack of knowledge of traffic legislation[12]. These factors explain why the risk of motorcycle accidents is substantially higher in this group[10].

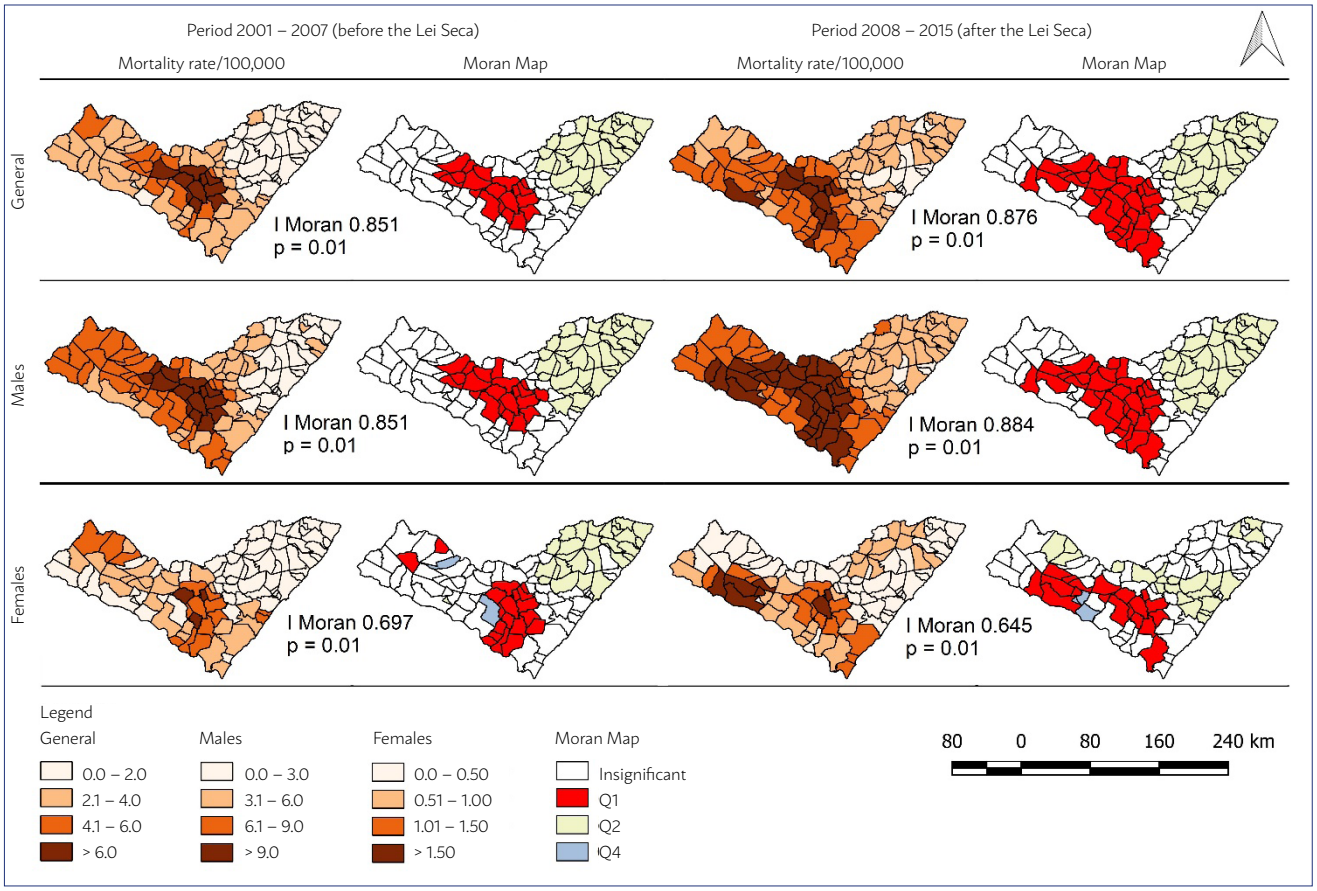
The prevalence of deaths in the mixed-race group can be explained by the Brazilian demographic characteristic since 43.1% of the population belongs to this race/ethnicity[13]. In addition, a study by Malta et al.[11] pointed out that, in the Brazilian population, mixed-race individuals wear a helmet less often than white individuals, both as the driver (82.1 and 87.9%, respectively) and as a passenger (78.1 and 86.4%, respectively). This points to the existence of sociocultural factors that determine the use of personal protective equipment.

In addition to all of these factors described, there has also been an increase in the number of motorcycles. In 2001, the number of two-wheeled vehicles in Alagoas was 33,209; in 2005, into was 58,579 (an increase of 76.3%)[14]. The epidemiological results of this entire context of vulnerabilities justify the upward trend in mortality, observed in our study between 2001 and 2007, with a percentage growth rate of 18.9% in the general population and 19.0% among males, as well as the geographic expansion of occurrences.

In addition to the proximal social determinants, it is pertinent to highlight those of a more distant sphere, i.e., the socioeconomic changes of the first decade of the 21st century, such as improved purchasing power, mainly in the North and Northeast regions of the country[15], the percentage of the population that left the poverty range[16], and the lower cost of motorcycles when compared to four-wheeled vehicles[15]. In addition, motorcycles assumed an important role in the dynamics of the Brazilian economy, since it has become an opportunity to scape unemployment[17].

The approval of the Brazilian Traffic Code, in

FIGURE 1



1997[18], did not have the expected effect; there was a reduction only in the first years when the number of deaths from traffic accidents dropped from 35,000 (1997) to 29,000 (2000)[19,20]. Due to the need for new mechanisms capable of making the Brazilian traffic safer, the *Lei Seca* (Law No. 11.705/2008) was created in an attempt to prevent drinking and driving[21]. A national study showed a less pronounced growth of mortality after it went into force[20], resembling the temporal pattern observed in Alagoas from 2008. In 2013, the decision 432 of the National Traffic Council lowered the levels of alcohol accepted in the blood-alcohol content test and raised the value of the fines for offenders[22], making the law even more severe.

It is also necessary to discuss the geographical distribution of these deaths and raise potential local factors associated with patterns of mortality. In this study, the areas of greatest risk were concentrated in the agreste and sertão in municipalities with a smaller population. Similar results were found in Pernambuco, where the highest mortality rates were in the interior of the state[23]. In addition, national research pointed to municipalities with up to 20,000 inhabitants and from 20,000 to 100,000 at higher risk of mortality in comparison to larger municipalities[23]. In Alagoas, only Maceió and Arapiraca have a population of over 200,000 inhabitants. In these municipalities, the determinant factors previously discussed are heightened by the absence of a state supervisory power[23].

CONCLUSION

The study showed consistent evidence that mortality from motorcycle traffic accidents is an important public health problem in the state of Alagoas, even after considering the positive impacts of the *Lei Seca*. We highlighted the male mortality and geographic expansion of deaths, which were concentrated on the interior of Alagoas (agreste and sertão regions).

Conflicts of interest:

None. No financial funding was received. This study was waived approval by the Research Ethics Committee.

Contribution of the authors

Carlos Dornels Freire de Souza; Leonardo Feitosa da Silva; Thiago Cavalcanti Leal; João Paulo Silva de Paiva: Participated in the development of the concept, planning of the study, data collection and analysis, discussion of the results, scientific writing, as well as in the review and approval of the final version of the work.

Michael Ferreira Machado; Maria Deysiane Porto de Araújo: Participated in the writing of the results, discussion, scientific writing, as well as in the review and approval of the final version of the work.

RESUMO

OBJETIVO: *Analisar o perfil epidemiológico e a distribuição espaço-temporal da mortalidade em acidentes motociclísticos em Alagoas antes (2001-2007) e após a lei seca (2008-2015).*

MÉTODOS: *Estudo ecológico misto. Foram incluídos no estudo todos os óbitos ocorridos no estado que tiveram como causa básica os códigos V20-V29 (CID-10). Foram analisadas as variáveis sociodemográficas e as taxas de mortalidade calculadas segundo sexo. Para a análise temporal, empregou-se o modelo de regressão por pontos de inflexão. Para análise espacial, as taxas foram suavizadas pelo Modelo Bayesiano Empírico Local e, posteriormente, foi empregada a estatística de Moran Global e Local para a identificação dos aglomerados espaciais de risco.*

RESULTADOS: *Foram registrados 1.458 óbitos em acidentes motociclísticos no período estudado, destacando-se: sexo masculino (91,29%), idade economicamente ativa (82,93%) e raça parda (78,12%). Na população masculina, verificou-se tendência de crescimento entre 2001 e 2007 (19,0%; p<0,001) e de declínio a partir de 2008 (−11,2%; p<0,001). A modelagem espacial mostrou que as áreas de maior risco de mortalidade estão situadas no agreste e sertão do estado (p=0,01).*

CONCLUSÃO: *A mortalidade em acidentes motociclísticos é um importante problema de saúde pública em Alagoas, com destaque para a mortalidade masculina e concentração geográfica no interior do estado.*

PALAVRAS-CHAVE: *Acidentes de trânsito. Estudos ecológicos. Registros de mortalidade.*

REFERENCES

1. World Health Organization (WHO). Global status report on road safety 2015: supporting a decade of action. Geneva: World Health Organization; 2015.
2. Beck LF, Dellinger AM, O'Neil ME. Motor vehicle crash injury rates by mode of travel, United States: using exposure-based methods to quantify differences. Am J Epidemiol. 2007;166(2):212-8.
3. Corgozinho MM, Montagner MA, Rodrigues MAC. Vulnerabilidade sobre duas rodas: tendência e perfil demográfico da mortalidade decorrente da violência no trânsito motociclístico no Brasil, 2004-2014. Cad Saúde Colet. 2018;26(1):92-9.
4. Morais Neto OL, Silva MMA, Lima CM, Malta DC, Silva Jr JB. Projeto Vida no Trânsito: avaliação das ações em cinco capitais brasileiras, 2011-2012. Epidemiol Serv Saude. 2013;22(3):373-82.
5. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo Demográfico 2010. [citado em 2019 Jan 25]. Disponível em: <https://www.ibge.gov.br>.
6. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med. 2000;19(3):335-51.
7. Santos SM, Souza WV. Introdução à estatística espacial para a saúde pública. Brasília: Ministério da Saúde; 2007.
8. Silva PLN, Santos AGP, Cruz PKR, Rocha JFD, Ferreira IR, Silva VF. Morbimortalidade de acidentes de trânsito envolvendo motocicletas: uma revisão de literatura. J Health Biol Sci. 2018;6(4):437-48.
9. Vasconcellos EA. Risco no trânsito, omissão e calamidade: impactos do incentivo à motocicleta no Brasil. São Paulo: Ed. do Autor; 2013.
10. Associação Brasileira dos Fabricantes de Ciclomotores, Motonetas, Bicicletas e Similares (Abraciclo). Anuário da Indústria Brasileira de Duas Rodas, 2012. [citado em 2019 Jan 25]. Disponível em: <http://www.abraciclo.com.br/anuario-2012>.
11. Malta DC, Andrade SSCA, Gomes N, Silva MMA, Morais Neto OL, Reis AAC, et al. Lesões no trânsito e uso de equipamento de proteção na população brasileira, segundo estudo de base populacional. Ciênc Saúde Colet. 2016;21(1):399-409.
12. Barros FHV, Silva LO, Roseno MASG, Olinda AG, Souza JBR, Amaral JJF. Prevalência dos acidentes de motocicleta envolvendo os adolescentes de Quixadá. Id on Line Rev Mult Psic. 2018;12(42 supl 1):511-24.
13. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo 2010. [citado em 2019 Jan 25]. Disponível em: https://censo2010.ibge.gov.br
14. Departamento Nacional de Trânsito (DENATRAN). Frota de Veículos 2019. [citado em 2019 Jan 25]. Disponível em: <https://www.denatran.gov.br/estatistica/237-frota-veiculos>
15. Rocha GS, Schor N. Acidentes de motocicleta no município de Rio Branco: caracterização e tendências. Ciênc Saúde Colet. 2013;18(3):721-31.
16. Instituto de Pesquisa Econômica Aplicada (IPEA). Mobilidade urbana e posse de veículos: análise da PNAD. 2009. Brasília: IPEA; 2010.
17. Silva DW, Andrade SM, Soares DA, Soares DFPP, Mathias TAF. Perfil do trabalho e acidentes de trânsito entre motociclistas de entregas em dois municípios de médio porte do Estado do Paraná, Brasil. Cad Saúde Pública. 2008;24(11):2643-52.
18. Brasil (BR). Lei nº 9.503, de 23 de setembro de 1997. Institui o Código de Trânsito Brasileiro. Diário Oficial da União 1997;24 set. [citado em 2019 fev 12]. Disponível em: http://www.planalto.gov.br/ccivil_03/LEIS/L9503.htm.
19. Vasconcellos EA. Road safety impacts of the motorcycle in Brazil. Int J Inj Contr Saf Promot. 2013;20(2):144-51.
20. Abreu DROM, Souza EM, Mathias TAF. Impacto do Código de Trânsito Brasileiro e da Lei Seca na mortalidade por acidentes de trânsito. Cad Saúde Pública. 2018;34(8):e00122117.
21. Brasil (BR). Lei nº 11.705, de 19 de junho de 2008. Altera a Lei nº 9.503, de 23 de setembro de 1997. Diário oficial da União 2008;20 jun.
22. Departamento Nacional de Trânsito (DENATRAN). Resolução nº 432/13. [citado em 2019 Fev 12]. Disponível em: https://www.denatran.gov.br/download/Resolucoes/(resolu%C3%A7%C3%A3o%20432.2013c).pdf
23. Silva PHNV, Lima MLC, Moreira RS, Souza WV, Cabral APS. Estudo espacial da mortalidade por acidentes de motocicleta em Pernambuco. Rev Saúde Pública 2011;45(2):409-15.

ORIGINAL ARTICLE

Metabolic syndrome in pregnancy and postpartum: prevalence and associated factors

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SUMMARY

OBJECTIVE: *Evaluate the prevalence of metabolic syndrome (MS) and the main associated maternal factors in women without pre-gestational conditions, in early pregnancy and in the immediate postpartum.*

METHODS: *Two hundred pregnant women were evaluated at the 16th week of pregnancy, and 187 were reassessed postpartum. MS was diagnosed according to the criteria by the National Cholesterol Education Program Adult Treatment Panel III. In addition to the diagnostic criteria, anthropometric measures, blood pressure, metabolic profile, and visceral and subcutaneous fat thickness (by ultrasonography) were collected from the pregnant woman. The student's t-test was used to compare the prevalence of MS and its components in the 16th week and in the postpartum. Multiple logistic regression was performed to identify the principal factors associated with the syndrome.*

RESULTS: *The prevalence of the MS was 3.0% in early pregnancy and 9.7% postpartum (p=0.01). Following multiple logistic regression, the prepregnancy body mass index (BMI) (p=0.04) and high-density lipoprotein cholesterol (HDL-c) (p=0.02) remained associated with MS at 16 weeks, and triglyceride levels evaluated in postpartum (p<0.001) with MS in postpartum.*

CONCLUSION: *The frequency of the MS was high in the immediate postpartum. The factors associated were prepregnancy BMI and HDL-c at the 16th week, as well as triglyceride levels postpartum.*

KEYWORDS: *Metabolic syndrome. Pregnancy. Postpartum period. Risk factors.*

INTRODUCTION

Metabolic syndrome (MS) is defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) as the presence of at least three of the following characteristics: abdominal obesity (abdominal circumference ≥88 cm), triglycerides ≥150 mg/dL, cholesterol, high-density lipoprotein cholesterol (HDL-c) <50 mg/dL, blood pressure (BP) ≥130/85 mmHg, and fasting glycemia ≥100 mg/dL[1]. Different criteria, however, are being used to classify the syndrome during pregnancy and in the postpartum period, when the abdominal circumference is significantly changed.[2-4]

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Despite the contradictions regarding classification in these periods, studies showed a prevalence of MS of 12.4% in pregnant women and 29% in puerperal women.[5,6] The factors often associated with the syndrome were age, parity, dyslipidemia, gestational diabetes mellitus (GDM), hypertension, and obesity.[5,6-9]

The high prevalence found by the studies, the metabolic risks from the physiological changes of pregnancy and the postpartum period, the lack of established parameters to classify the syndrome, and the inconsistencies regarding its prevalence and associated factors in women with no previous diseases suggest the need for research on the topic. Thus, the objective of this study was to evaluate the prevalence of MS and the main maternal factors associated with it in women without pre-gestational diseases, at the beginning of pregnancy and in the immediate postpartum period.

METHODS

Study design

This is a cohort study with 200 pregnant women treated in basic health units of the city of Campina Grande, Paraíba, Brasil. The women were evaluated in the 16th week of gestation (n=200) and re-evaluated during the immediate postpartum period (n=187) in the *Instituto Paraibano de Pesquisa Professor Joaquim Amorim Neto* (IPESQ), between September 2014 and December 2015. The research was approved by the Research Ethics Committee of the Federal University of Paraíba (CAAE: 03649512.9.0000.5182), and all participants signed an informed consent form (ICF).

Sample Size

The sample size was calculated by the OpenEpi, version 2.3 (Atlanta, GA, USA), assuming a rate of waist circumference increase of 22.8% in the immediate postpartum.[6] For a 85% power and a 95% confidence level, 148 pregnant women would have to be included. This number was increased by 30% to compensate for possible losses during follow-up.

Eligibility criteria

We included pregnant women, confirmed by ultrasound, with gestational age less than or equal to 16 weeks. We excluded women with pre-gestational diabetes mellitus (DM), suffering from psychiatric disorders, chronic maternal diseases (hypertension, heart disease, kidney disease, epilepsy, kidney failure), congenital malformations, and multiple pregnancies.

Data collection and procedures performed

In the 16th week of gestation (± 1 week), after signing the ICF, the women underwent an ultrasound to confirm their pregnancy and answered a questionnaire with information about biological, sociodemographic, and obstetric characteristics. Next, we evaluated the diagnostic criteria for MS, its prevalence, and the possibly related maternal risk factors.

MS was diagnosed according to the classification by the NCEP/ATP III[1], and the maternal factors investigated were the thicknesses of visceral and subcutaneous fat, anthropometric measurements, blood pressure (BP), and metabolic profile. After these assessments, the pregnant women were scheduled to return at the 28th week and the immediate postpartum period (≤10 days after delivery). In the 28th week, we carried out only the oral glucose tolerance test (OGTT) and, in the postpartum period, were repeated all previous assessments.

The ultrasound was carried out by the same professional, a specialist in fetal medicine, who also measured the thickness of visceral and subcutaneous fat, according to the technique described by Armellini et al.[10]. The maternal BP was measured by the recommended palpation and auscultation methods.[11]

The anthropometric assessment included weight, height, body mass index (BMI), weight gain, circumferences of the waist, arm, and thigh, and the triceps and suprailiac skinfolds. Based on their BMI, the women were classified as underweight, average weight, overweight, or obese.[12] The weight gain was obtained from the difference between the women's weight in each of these moments and their pre-gestational weight self-reported during the filling out of the questionnaire. The circumferences of the waist, arm, and thigh, and the skinfolds were evaluated according to Jackson and Pollock.[13]

The biochemical measurements included total cholesterol, HDL-c, low-density lipoprotein cholesterol (LDL-c), triglycerides, insulin, homeostasis model assessment (HOMA-IR), and levels of fasting glucose. Insulin resistance was determined according to the HOMA-IR [fasting insulin (mUI/ml) x fasting glucose (mmol/l)/22.5].[14]

At the 28th week of gestation, the women returned to the PESQ to undergo the OGTT, as previously scheduled. The diagnosis of gestational diabetes mellitus (GDM) was based on the confirmed fasting glycemia ≥92 mg/dl at the 16th week of gestation, in addition to an OGTT with any of the following abnormal values:

fasting glycemia ≥92 mg/dl; one-hour levels ≥180 mg/dl, and two-hour levels ≥153 mg/dl.[14]

Data Analysis

Medcalc, version 15.6.1. (Medical Software bvba, Ostend, Belgium), and Epi Info, version 7.1.5 (Atlanta, GA, USA) were used for the statistical analysis. The Student t-test was used to compare the prevalence of metabolic syndrome and its components in these periods. A bivariate analysis was then performed to test the association between the variables studied and MS (ANOVA and Kruskal-Wallis Test). Stepwise logistic regression was performed to identify the main maternal factors associated with the syndrome, including the variables that remained associated with a significance level of 20% in the bivariate analysis. The final model included only the variables that remained associated with the outcomes at a significance level of 5%.

RESULTS

The prevalence of MS at the 16th week was 3.0% (n=6) in comparison to 9.7% (n=18) during the immediate postpartum period (p=0.01). Regarding the criteria for MS classification at the 16th week, the frequency of abdominal obesity (abdominal circumference≥88 cm), HDL-c <50mg, triglycerides ≥150mg/L, fasting glycemia ≥100mg/dL, and BP ≥130/85mmHg was 100% and 34% (p<0.001), 83.3% and 40.7% (p=0.02), 83.3% and 11.8% (p<0.001), 0% and 0.5% (p=0.84), and 0% and 1% (p=0.93) in the groups with and without the syndrome, respectively (Table 1). During the immediate post-partum period, the frequencies were 77.8% and 40.8% (p<0.001); 88.9% and 39% (p<0.001); 83.3% and 11.2% (p<0.001); 0% and 1.2% (p=0.84); 16.6% and 2.9% (p=0.01), in both groups (Table 1).

The mean age in the groups with and without MS were 31.5±8.4 and 25.4±5.7 years at the 16th week of gestation (p=0.01). In the postpartum period, these averages were 27.0±7.8 and 25.6±5.6 years in both groups, respectively (p=0.38). With respect to formal education, in both periods, the averages were 6.6±3.0 and 9.7±3.8 years at the 16th week, and 8.8±3.2 and 9.8±3.9 years in the postpartum period for the groups with and without the syndrome (p=0.05; p = 0.32) (Table 2).

Regarding the reproductive history of the women, those with MS at the 16th week of pregnancy had an average of 3.8±2.3 pregnancies, 0.0±0.5 abortions, and 2.5±2.3 deliveries compared with 2.0±1.2 pregnancies, 0.2±0.5 abortions, and 0.8±1.1 deliveries by the women without the syndrome (p=0.01; p=0.46; p<0.001, respectively). In the postpartum period, the averages were of 2.7±1.6 pregnancies, 0.3±0.6 abortions, and 1.4±1.6 deliveries in the group with MS, while in the group without the syndrome the averages were 2.0±1.1 pregnancies, 0.2±0.5 abortions, and 0.7±0.9 deliveries (p=0.02; p=0.82; and p=0.04, respectively). The average interval between pregnancies, at the 16th week, was 4.2±2.3 and 3.5±3.3 years in both groups (p=0.64), at the same time, compared to 3.5±2.4 and 3.4±3.4 years (p=0.89) in the postpartum period (Table 2).

As to the nutritional status of the women, 13.0% (n=26) were classified with low weight at the 16th week of gestation, compared to 1.3% (n=2) in the postpartum period (p<0.001); 48.7% (n=91) and 57.3% (n=107) were classified as eutrophic in both periods (p=0.20), and the women considered overweight or obese were 38.1% (n=76) and 41.3% (n=77) (p=0.62), respectively.

The average maternal weight in the groups with and without the syndrome was 72.4±15.4 kg and 62.4±11.2 kg (p=0.03) at the 16th week of gestation, while in the postpartum period, it was 68.7 ± 15.1 kg and 65.0±10.3 kg (p<0.20) (Table 3). The mean BMI was 28.3±5.1 and 24.5±4.0 (p<0.001) at the 16th week of gestation when compared to 28.3±3.0 and 25.7±4.1 (p=0.01) in the postpartum period (Table 2). There was

TABLE 1. DIAGNOSTIC CRITERIA FOR MS, ACCORDING TO THE NCEP/ATP III CLASSIFICATION, IN WOMEN WITH AND WITHOUT THE SYNDROME. CAMPINA GRANDE, PARAÍBA, BRASIL, 2015.

	MS at the 16th week (n=200)			MS in the immediate postpartum period (n=187)			p#
	Yes %	No %	p-value	Yes % (n)	No % (n)	p-value*	
Abdominal circumference ≥88cm	100 (6)	34 (66)	<0.001	77.8 (14)	40.8 (69)	<0.001	0.02
HDL-c (mg/dl) <50mg/dL	83.3 (5)	40.7 (79)	0.02	88.9 (16)	39 (66)	<0.001	0.49
Triglycerides (mg/dl) ≥150mg/dL	83.3 (5)	11.8 (23)	<0.001	83.3 (15)	11.2 (19)	<0.001	0.05
Fasting blood glucose (mg/dl) ≥ 100mg/dL	0 (0)	0.5 (1)	0.84	0 (0)	1.2 (2)	0.84	0.92
BP ≥130/85mmHg	0 (0)	1 (2)	0.93	16.6 (3)	2.9 (5)	0.01	0.15

MS: Metabolic syndrome; HDL-c: high-density lipoprotein cholesterol; BP: Blood pressure; *ANOVA. # Student's t-test.

TABLE 2. BIOLOGICAL, ANTHROPOMETRIC, AND SOCIODEMOGRAPHIC CHARACTERISTICS, VISCERAL AND SUBCUTANEOUS FAT, AND METABOLIC PROFILE OF WOMEN WITH AND WITHOUT MS. CAMPINA GRANDE, PARAÍBA, BRASIL, 2015.

	MS at the 16th week (n=200)			MS in the immediate postpartum period (n=187)			p
	Yes %	No %	p	Yes %	No %	p	
Age	31.5 ±8.4	25.4 ±5.7	0.01	27.0 ±7.8	25.6 ±5.6	0.38	0.80
Formal education	6.6 ±3.0	9.7 ±3.8	0.05	8.8 ±3.2	9.8 ±3.9	0.32	0.02
Pregnancies	3.8 ± 2.3	2.0 ± 1.2	0.01#	2.7 ± 1.6	2.0 ± 1.1	0.02#	0.79
Abortions	0.0 ± 0.5	0.2 ± 0.5	0.46#	0.3 ± 0.6	0.2 ± 0.5	0.82#	0.79
Birth interval	4.2 ± 2.3	3.5 ± 3.3	0.64	3.5 ± 2.4	3.4 ± 3.4	0.89	0.07
Parity	2.5 ± 2.3	0.8 ± 1.1	<0.001#	1.4 ± 1.6	0.7 ± 0.9	0.04#	0.23
Visceral Fat	5.9 ±1.2	5.2±1.3	0.20	5.5 ±1.9	5.6 ±1.5	0.83	0.83
Subcutaneous fat	2.9 ±0.8	2.3 ±0.8	0.07	3.0 ±1.0	2.4 ±0.8	0.01	0.01
Systolic BP	116.8 ± 4.6	112.5 ± 10.4	0.35	123.4 ± 17.8	115.0±11.0	0.02	0.02
Diastolic BP	66.8 ±14.1	68.2 ±9.6	0.74	81.0 ±12.2	74.4±10.4	0.04	0.04
Pre-gestational BMI	29.0 ±5.7	24.4 ±4.3	0.01	28.0 ±5.7	24.1 ±4.0	p<0.00	0.02
Height	1.5 ±0.0	1.5 ±0.6	0.18	1.5 ±0.6	1.8 ±0.6	0.63	0.13
Weight	72.4 ±15.4	62.4 ±11.2	0.03	68.7 ± 15.1	65.2 ± 10.3	0.20	0.20
BMI	28.3 ±5.1	24.5 ±4.0	<0.001	28.3 ±3.0	25.7 ±4.1	0.01	<0,001
Weight gain	14.4 ±12.6	208.3 ±8.3	0.27	1.1 ±6.9	4.8 ±4.9	<0.001	0.03
Waist circumference	98.9 ± 12.4	88.0 ± 9.6	<0.001	98.6 ±8.5	91.9 ±9.5	<0,001	<0,001
Arm circumference	34.1 ±6.3	28.0 ±3.4	<0,001	29.4 ±2.3	28.5 ±3.1	0.13	0.13
Thigh circumference	30.8 ±11.1	29.6 ±7.7	0.72	41.7 ±10.1	38.2 ±9.2	0.21	0.21
Suprailiac fold	30.3±16.4	22.8 ±8.9	0.05	26.2 ±7.8	22.6 ±6.0	0.03	0.05
Triceps fold	28.1 ±11.5	21.3 ±5.6	<0.001	26.2 ±7.8	22.6 ±6.0	0.03	0.03
Total cholesterol	195.3±10.0	168.6±30.9	0.03	209.4±47.4	215.0±42.6	0.62	0.62
Fasting glucose	67.5 ±4.4	70.2 ±15.5	0.66	73.0 ±11.7	69.3 ±8.6	0.12	0.61
Insulin	9.3 ±5.8	5.7 ±4.0	0.03	7.1 ±4.9	5.5 ±3.6	0.13	0.13
HDL-c	50.5 ±11.2	45.0 ±2.5	0.23	49.8±10.5	39.0 ± 6.5	<0.001	<0,001
LDL-c	106.6 ±16.7	96.8 ±24.8	0.32	121.7±46.5	143.0±39.6	0.06	0.06
Triglycerides	218.1 ±51.7	108.5 ±53.1	<0,001	245.0 ±92.3	111.5±22.3	<0.001	<0,001
HOMA-IR	24.2±19.8	14.3±14.4	0.08	24.2±22.1	17.4±12.0	0.05	0.05

MS: Metabolic syndrome; BMI: body mass index; GDM: gestational diabetes mellitus; BP: Blood pressure; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HOMA-IR: model for the evaluation of homeostasis; IQR: interquartile interval. Values were expressed as mean± standard deviation. * Student's t-test. # Kruskal Wallis test.

an association between maternal weight gain and the presence of MS (p<0.001).

The average thickness of visceral fat was 5.9±1.2cm and 5.2±1,3cm (p=0.20) at the 16th week, while in the immediate post-partum period, it was 5.5 ± 1.9cm and 5.6±1.5cm (p=0.83) in the groups of women with and without MS (Table 2). No association was found between the thickness of visceral fat and the syndrome (p=0.88). The mean subcutaneous fat thickness was 2.9±0.8 and 2.3±0.8 (p= 0.07) at the 16th week when compared to 3.0±1.0 and 2.4±0.8 (p=0.01) in the post-partum period, in both groups, respectively (Table 2). An association was found between the subcutaneous fat and the presence of MS (p<0.001).

TABLE 3. FINAL LOGISTIC REGRESSION MODEL FOR FACTORS ASSOCIATED WITH MS IN THE 16TH WEEK OF GESTATION AND IN THE IMMEDIATE POST-PARTUM PERIOD. CAMPINA GRANDE, PARAÍBA, BRASIL, 2015.

	Odds Ratio	95% CI	Coefficient	P-value*
16th week (n=200)				
Pre-gestational BMI	1.08	1.00-1.17	0.08	0.04
HDL-c	1.02	1.00-1.04	0.02	0.02
Immediate postpartum (n=187)				
Triglycerides (evaluated in the immediate puerperium)	1.04	1.01-1.07	0.04	<0.001

* Stepwise logistic regression. 95% CI: 95% confidence interval; BMI: body mass index; HDL-c: high-density lipoprotein cholesterol.

Compared to the 16th week of gestation, in the postpartum period, there was a greater proportion of women that presented increased levels of fasting glucose (0.5% and 1.3%) (p=0.32), triglycerides (16.3% and 22.6%) (p=0.60), total cholesterol (16.3% and 63.2%) (p=0.21), and LDL-c (44.4% and 84.8%) (p=0.20) in the groups with and without MS. After analyzing both periods, the proportion of women with low levels of HDL-c increased slightly in the postpartum (58.2%) when compared with the 16th week (57.0%) (p=0.03). The Frequency of GDM was 2.8% (n=5).

The mean levels of total cholesterol were 195.3±10.0 mg/dl and 168.6±30.9 mg/dl (p=0.03) in women with and without metabolic syndrome at the 16th week of gestation and 209.4±47,4mg/dl and 215.0±42.6 (p=0.62), simultaneously, in the postpartum period. The average of fasting glucose, after comparing both groups, were 67.5±4.4mg/dl and 70.2±15.5 mg/dl (p=0.66) at the 16th week, while in the post-partum period, they were 73.0±11.7mg/dl and 69.3±8.6 mg/dl (p=0.12) (Table 2).

The average levels of insulin were 9.3±5.8 mUI/ml and 5.7±4.0 mUI/ml (p=0.03) for women with and without MS at the 16th week of gestation, and 7.1±4.9mUI/ml and 5.5±3.6 mUI/ml (p=0.13), respectively, in the postpartum period. Regarding the triglyceride levels in both groups, the averages were 218.1±51,7mg/dl and 108.5±53.1 (p<0.001) at the 16th week, and 245.0±92,3mg/dl and 111.5±22.3 (p<0.001) post-partum (Table 2). As for resistance to insulin, the mean HOMA-IR was 24.2±19.8 and 14.3±14.4 (p=0.08) at the 16th week of gestation, while during the post-partum period it was 24.2±22.1 and 17.4±12.0 (p=0.05) in the groups with and without MS. (Table 2).

After logistic regression analysis, the pre-gestational BMI (p=0.04) and HDL-c (p=0.02) remained significantly associated with MS in the 16th week of gestation. In the postpartum period, only the levels of triglycerides, analyzed in this period, remained significantly associated with the syndrome (p<0.001) (Table 3).

DISCUSSION

The prevalence of MS in the immediate postpartum was high when compared to that found at the beginning of pregnancy, and the only factors that remained associated with the syndrome were pre-gestational BMI, HDL-c at the 16th week, and triglycerides in the postpartum period.

A similar frequency of the syndrome in early pregnancy was reported in a study involving women at 12 weeks of gestation.[15] However, a retrospective research with women at 14 to 16 weeks of gestation reported a much higher prevalence (12.3%).[5] This difference, however, may have resulted from the application of different criteria for the classification of MS. In fact, the researchers in that multicenter study used the parameters by the International Diabetes Federation (FID), which establish a cutoff point for waist circumference (80 cm) lower than that applied in this study[5]. Since there is a physiological change to that measurement during pregnancy, an even lower cutoff point could predispose more easily a high rate of MS diagnoses.

Postpartum studies also showed higher prevalence rates of MS in comparison to our results, reaching 39%[4,6,9,15-17]. However, this difference may be explained by the selection of the sample, since our study excluded pregnant women with associated morbidities because the objective was to understand the magnitude of the disease in pregnant women without pre-gestational diseases.

With respect to the weight and nutritional status of the pregnant women, those with MS presented excess weight, and the pre-gestational BMI remained significantly associated with MS in the 16th week. Unlike our results, other studies showed an association between pre-gestational BMI indicative of overweight and obesity and the presence of MS in the postpartum period.[9,16,18] In these studies, however, MS was diagnosed in the postpartum period in women with a diagnosis of GDM who, according to the pre-gestational BMI, were overweight/obese and whose levels of fasting glucose were high.[9,16,18]

In relation to the biochemical analysis, only the levels of HDL-c in the 16th week and the triglyceride levels in the postpartum period remained significantly associated with MS in this study. Corroborating these findings, a study carried out at the beginning of the gestation showed higher average levels of triglycerides (192.5±87.5 mg/dl and 105±61.2 mg/dl) and lower HDL-c (456.79±114.2 mg/dl and 532.92±152.26 mg/dl) in women with MS in comparison to those without it.[4]

Other studies have reported conflicting results, with findings of a correlation between MS and insulin resistance[15] and between MS and hypertension;[6,16,19] however, unlike in the present study,

pregnant women with GDM and women with various degrees of glucose intolerance were included in these samples.

A limitation of this study is the risk of an overestimated prevalence of MS due to the difficulty in classifying it in both periods through the pre-established criteria. The strengths of this study are the homogeneity of the sample, the small number of losses, and the inclusion of healthy women.

Studies on metabolic syndrome and its associated risk factors in pregnant women can assist in predicting the disease during pregnancy and in the postpartum period, allowing the use of preventive strategies to reduce maternal and child damage in the short and long term.

CONCLUSION

The frequency of metabolic syndrome was higher during the immediate postpartum period, and a pre-gestational BMI indicative of overweight/obesity and abnormal levels of HDL-c at the 16th week of pregnancy, in addition to abnormal levels of triglycerides in the immediate post-partum period, seem to represent important risk factors for the development of metabolic syndrome in these periods.

No conflicts of interest. The study was approved in the Ethics and Research Committee by the CAAE: 03649512.9.0000.5182, on the 26/03/2014.

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RESUMO

OBJETIVO: *Avaliar a prevalência da SM e os principais fatores maternos associados em mulheres sem doenças pré-gestacionais, no início da gravidez e no pós-parto imediato.*

MÉTODOS: *Foram avaliadas 200 mulheres na 16ª semana de gravidez, sendo 187 reavaliadas no pós-parto. A SM foi diagnosticada de acordo com os critérios do National Cholesterol Education Program Adult Treatment Panel III. Além dos critérios diagnósticos da síndrome, foram coletadas medidas antropométricas, pressão arterial, perfil metabólico e espessura de gordura visceral e subcutânea (através de ultrassonografia) da gestante. O teste t de Student foi usado para comparar a prevalência de SM e dos seus componentes nos dois momentos. Os modelos de regressão logística múltipla, para investigar os principais fatores associados à síndrome na 16ª semana de gestação e no pós-parto.*

RESULTADOS: *A prevalência da SM foi de 3,0% no início da gravidez e 9,7% no pós-parto (p=0,01). O índice de massa corporal (IMC) pré-gravídico (p=0,04) e o colesterol lipoproteínas de alta densidade (HDL-c) (p=0,02) permaneceram associados à SM na 16ª semana. Após o parto, os níveis de triglicerídeos permaneceram associados à SM no pós-parto (p<0,001).*

CONCLUSÃO: *A prevalência da SM foi alta no pós-parto imediato e os fatores associados à síndrome foram IMC pré-gravídico e HDL-c na 16ª semana, e níveis triglicerídeos no pós-parto.*

PALAVRAS-CHAVES: *Síndrome metabólica. Gravidez. Período pós-parto. Fatores de risco.*

REFERENCES

1. Carson MP. Society for maternal and fetal medicine workshop on pregnancy as a window to future health: clinical utility of classifying women with metabolic syndrome. Semin Perinatol. 2015;39(4):284-9.
2. Hakkarainen H, Huopio H, Cederberg H, Voutilainen R, Heinonen S. Future risk of metabolic syndrome in women with a previous LGA delivery stratified by gestational glucose tolerance: a prospective cohort study. BMC Pregnancy Childbirth. 2018;18(1):326.
3. Varner MW, Rice MM, Landon MB, Casey BM, Reddy UM, Wapner RJ, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network. Pregnancies after the diagnosis of mild gestational diabetes mellitus and risk of cardiometabolic disorders. Obstet Gynecol. 2017;129(2):273-80.
4. Cho NH, Ahn CH, Lua JH, Kwak SH, Choi SH, Lim S, et al. Metabolic syndrome independently predicts future diabetes in women with a history of gestational diabetes mellitus. Medicine (Baltimore). 2016;95(35):e4582.
5. Grieger JA, Grzeskowiak LE, Smithers LG, Bianco-Miotto T, Leemaqz SY, Andraweera P, et al.. Metabolic syndrome and time to pregnancy: a retrospective study of nulliparous women. BJOG. 2019;126(7):852-62.
6. Rice MM, Landon MB, Varner MW, Casey BM, Reddy UM, Wapner RJ, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network (MFMU). Pregnancy-associated hypertension in glucose-intolerant pregnancy and subsequent metabolic syndrome. Obstet Gynecol. 2016;127(4):771-9.
7. Lee Y, Lee HN, KIM SJ, Koo J, Lee KE, Shin JE. Higher parity and risk of metabolic syndrome in Korean postmenopausal women: Korea National Health and Nutrition Survey 2010-2012. J Obstet Gynecol Res. 2018;44(11):2045-52.
8. Szostak-Węgierek D, Waśkiewicz A, Piotrowski W, Stepaniak U, Pająk A, Kwaśniewska M, et al. Metabolic syndrome and its components in Polish women of childbearing age: a nationwide study. BMC Public Health. 2017;18(1):15.

9. Puhkala J, Raitanen J, Kolu P, Tuominen P, Husu P, Luoto R. Metabolic syndrome in Finnish women 7 years after a gestational diabetes prevention trial. BMJ Open. 2017;7(3):e014565.

10. Armellini F, Zamboni M, Robbi R, Todesco T, Rigo L, Bergamo-Andreis IA, et al. Total and intra-abdominal fat measurements by ultrasound and computerized tomography. Int J Obes Relat Metab Disord. 1993;17(4):209-14.

11. American College of Obstetricians and Gynecologists. Hypertension in pregnancy: executive summary. Obstet Gynecol. 2013;122(5):1122-31.

12. Atalah E, Castillo C, Castro R, Aldea A. Proposal of a new standard for the nutritional assessment of pregnant women. Rev Med Chil. 1997;125(12):1429-36.

13. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. 1978. Br J Nutr. 2004;91(1):161-8.

14. Standards of Medical Care in Diabetes-2017: summary of revisions. Diabetes Care. 2017;40(Suppl 1):S4-S5.

15. Horvath B, Bodecs T, Boncz I, Bodis J. Metabolic syndrome in normal and complicated pregnancies. Metab Syndr Relat Disord. 2013;11(3):185-8.

16. Nouhjah S, Shahbazian H, Shahbazian N, Jahanfar S, Jahanshahi A, Cheraghian B, et al. Early postpartum metabolic syndrome in women with or without gestational diabetes: results from Life after Gestational Diabetes Ahvaz cohort study. Diabetes Metab Syndr. 2018;12(3):317-23.

17. Huvinen E, Eriksson JG, Koivusalo SB, Grotenfelt N, Tiitinen A, Stach-Lempinen B, et al. Heterogeneity of gestational diabetes (GDM) and long-term risk of diabetes and metabolic syndrome: findings from the RADIEL study follow-up. Acta Diabetol. 2018;55(5):493-501.

18. Barquiel B, Herranz L, Hillman N, Burgos MÁ, Pallardo LF. Prepregnancy body mass index and prenatal fasting glucose are effective predictors of early postpartum metabolic syndrome in Spanish mothers with gestational diabetes. Metab Syndr Relat Disord. 2014;12(9):457-63.

19. Yang JJ, Lee SA, Choi JY, Song M, Han S, Yoon HS, et al. Subsequent risk of metabolic syndrome in women with a history of preeclampsia: data from the Health Examinees Study. J Epidemiol. 2015;25(4):281-8.

REVIEW ARTICLE

Intra-tumor genetic heterogeneity in Wilms tumor samples

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SUMMARY

Childhood renal tumors account for ~7% of all childhood cancers, and most cases are embryonic Wilms' tumors (WT). Children with WT are usually treated by either COG or SIOP. The later treats the children using preoperative chemotherapy, but both have around 90% of overall survival in five years. WT is a genetically heterogeneous group with a low prevalence of known somatic alterations. Only around 30% of the cases present mutation in known genes, and there is a relatively high degree of intra-tumor genetic heterogeneity (ITGH). Besides potentially having an impact on the clinical outcome of patients, ITGH may interfere with the search for molecular markers that are prospectively being tested by COG and SIOP. In this review, we present the proposal of the current UMBRELLA SIOP Study 2017/Brazilian Renal Tumor Group that requires the multi-sampling collection of each tumor to better evaluate possible molecular markers, as well as to understand WT biology

KEYWORDS: *Wilms tumor. Biomarkers. Genetic Heterogeneity*

INTRODUCTION

Childhood renal tumors account for ~7% of all childhood cancers, and most cases are Wilms tumors (WT) or nephroblastomas (~90%), affecting one in 10,000 children under the age of fifteen[1]. It is estimated there are 500 new cases of WT every year in Brasil, with a median age-adjusted incidence rate of 9.5 cases per million[2]. The peak incidence is between the ages of 2 and 3 years[1], but bilateral cases and those associated with congenital syndromes (5 to 10% of the cases) are diagnosed earlier[3].

Non-WT renal tumors include clear cell sarcoma of the kidney (CCSK, 2-3%, 1% of the cases), with

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similar age presentation to WT, malignant rhabdoid tumor of the kidney (MRTK, 2-3%, 1% of the cases), which presents a peak incidence at 10 to 18 months, renal cell carcinoma (RCC, 1% of the cases), with a peak incidence among adolescents, benign congenital mesoblastic nephroma (CMF, 2-3% of the cases), and all other renal tumors, which include sarcoma and neuroectodermal tumor of the kidney, comprise 2-3% of the cases[4]. All renal tumors, but CMF, which is usually treated with surgery alone, have a poorer prognosis compared to WT. Due to its relatively higher incidence, this review will focus on WT.

Children with renal tumors are usually treated according to one of the two major clinical groups. The SIOP-RTSG (*Societe Internationale d'Oncologie Pediatrique - Renal Tumor Study Group*) protocol recommends children be treated with pre-operative chemotherapy; and the COG (*National Wilms Tumor Study/ Children's Oncology Group*) protocols advise children to be submitted to upfront surgery, with both reaching 90% of overall survival[5,6].

GENETIC EVENTS AND POTENTIAL PROGNOSTIC MARKERS FOR WILMS TUMORS

Most pediatric tumors present a short time that precedes the diagnosis, thus resulting in fewer evolutionary events compared to adult cancers[7]. Consistently, the mutational spectrum described in most pediatric solid tumors has been considerably small, including in WT. WT is a genetically heterogeneous group that presents a low prevalence of known somatic alterations and a relatively high degree of Intra-Tumor Genetic Heterogeneity (ITGH)[8,9]. For instance, 30% of WT cases have a known mutated gene, with *WT1*, *CTNNB1*, and *AMER1* being the most prevalent[10,11]. Moreover, alterations in microRNA processor genes (*DROSHA, DGCR8*, *DICER1*, and *TARBP2*) and *SIX1/2* were also found in up to 15% of WT[12-15]. The mutation spectrum of WT was properly discussed by other studies[4-11]. For the purpose of this review, we considered of interest the somatic mutations in *TP53* found in ~70% of the diffuse anaplastic WT and gain of *MYCN* found in up to 4% of the cases. Diffuse anaplastic tumors comprise 5% of WT cases. They present an unstable genome, but tumors that also had *TP53* mutations or loss presented a higher number of copy number alterations[14,16]. In diffuse anaplastic WT, mutation/loss in *TP53* was suggested to be an independent poor prognostic factor[16]. *MYCN* gain was associated with anaplasia and with poorer relapse-free and overall survival, independently of tumor histology[17]. A gain of 1q is found in up to 30% of WT cases and was considered a potential prognostic biomarker regardless of the treatment protocol (COG or SIOP). A gain of 1q was associated with poorer event-free and overall survival, and, if validated, it could be used to select patients who were first treated with surgery or chemotherapy for more aggressive treatment[18-20].

Loss of heterozygosity (LOH) of both 1p and 16q were associated with lower event-free and overall survival in WT treated with surgery first[21,22]. Following these findings, for the first time, the presence of molecular alterations was used to direct therapy in WT. COG intensified the treatment for stage III/IV WT, with loss of heterozygosity (LOH) of 16q, and 1p significant improving the event-free survival[23].

All potential biomarkers discussed here were studied in a single sample from each case without considering the existence of ITGH in WT.

Intra-tumor genetic heterogeneity in Wilms Tumor: the importance of prognostic markers

WT develops from primitive renal cells incapable of completing kidney differentiation, which results in a tumor that recapitulates nephrogenesis, with morphology, methylation, and gene expression similar to the fetal kidney[24-28]. WT is composed of varying proportions of three morphologically distinct cell types: undifferentiated blastemal cells, epithelial cells ordered into primitive structures, and stromal cells, which are related to the clinical behavior[29,30].

Intratumoral diversity is relatively common in chemotherapy-treated primary childhood cancers, even for WT that usually is described as presenting a relatively stable genome. Most WT subclones have low-frequency aberrations, but potential drivers may emerge as part of the ITGH, such as copy number neutral imbalance of 11p and trisomy 8, 1q gain, and 1p/16q loss[9].

The gain of 1q is a promising biomarker for patients with WT stratification into risk groups, although it can be an early or late event. In the latter scenario, for prospective studies that underlie clinical trials, the assessment of the ITGH by multisampling the tumor for proper evaluation of this biomarker is of utmost importance. Based on an analysis of 20

cases of WT, it was estimated the need for at least three tumor samples for each case[31].

Other alterations were characterized as ITGH in WT, such as *AMER1 (WTX)*[32], *DROSHA*, *SIX1*[33], and *TP53*[34]. These studies are still in early stages, and definitive interpretation and conclusion will only be possible after studying large multi-sampled tumor cohorts.

Proposal of the current SIOP protocol/ Brazilian Renal Tumor Group

There is an increasing effort to identify prognostic molecular markers for patients with WT. The current approach to risk stratification has reached the limits of what can be achieved through combinations of clinical and pathological features. The biological mechanisms involved in tumor treatment response still need to be uncovered and associated with the current risk factors to further improve prediction of each child's risk of relapse. Retrospective studies from SIOP and COG pointed to alterations that are being explored prospectively in patients with WT. However, any potential prognostic markers require prospective validations that consider the underlying ITGH of each case.

The current protocol recommends the collection of three tumor samples at different sites of the tumor and the adjacent normal kidney by the pathologist responsible for the diagnosis at the time of surgery. The area of the research sample must be removed from the region immediately adjacent to the diagnostic sample (paraffin block), avoiding areas of necrosis and hemorrhage (Figure 1A). It is extremely important to correctly identify each tube and record patient data to enable later association of experimental findings with the clinical-pathological characteristics. The tubes should be labeled as tumor (TW1, TW2, TW3, and so on) and kidney (NK1, NK2) samples for proper handling at the biomarker research stage. Note that the normal counterpart for comparison with WT is the renal cortex and not the kidney medulla. Samples may be used for DNA, RNA, and/or protein satisfactorily.

Samples should be collected immediately after surgery, frozen in liquid nitrogen, and sent to the Biobank of the institution for correct storage (Figure 2). For institutions without a Biobank, it is possible to ask for a tube with an appropriate reagent for tissue storage that keeps the tissue viable for further experiments. If this is the case, recommendations are: 1. the sample should be completely submerged by the reagent; 2. one of the dimensions of the tissue should be smaller than 0.5 cm to enable rapid stabilization of the molecules; 3. the approximate ideal ratio is 1:10, equivalent to 10 mg of tissue to 100 uL of reagent. Each tube contains between 800 and 1000 uL of reagent. Samples will be safe if stored at 37 °C for 24 hours; 15-25 °C for one week; 4 °C for six months; - 20 or -80 °C indefinitely. These tubes are provided only by the project coordinator and should be requested to the SIOP-BRTG via SOBOPE (Figure 1B). In one year, the BRTG received eighteen cases from six institutions with at least three tumor and

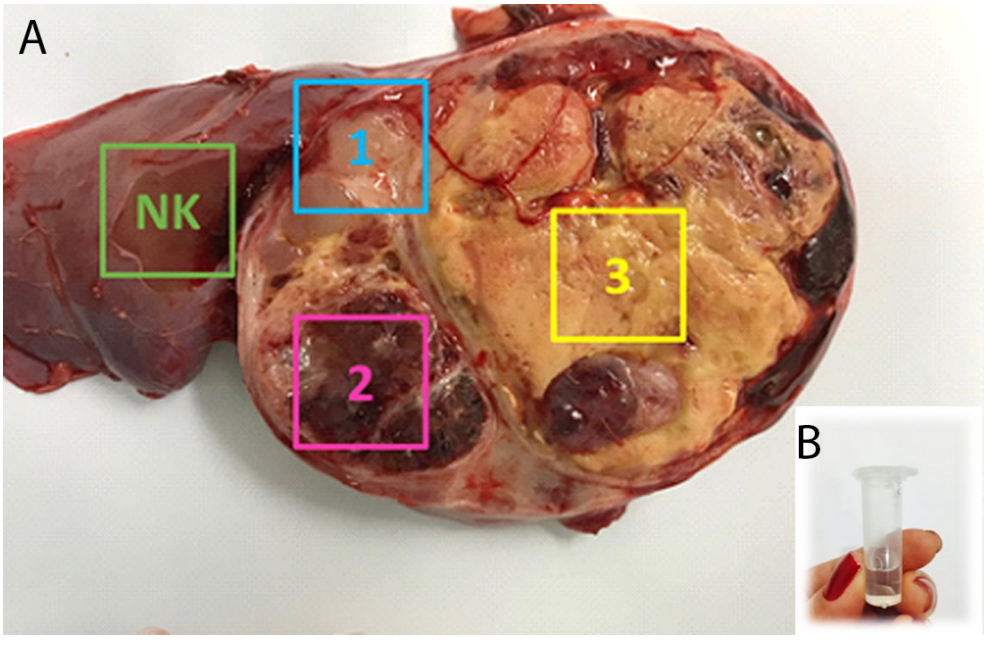

FIGURE 1. A. NEPHRECTOMY SPECIMEN. SAMPLES WERE COLLECTED FROM THREE TUMOR AREAS (1, 2, 3) AND ADJACENT NORMAL KIDNEY (NK) BY THE PATHOLOGIST. B – SCHEME FOR SAMPLE COLLECTION. TUBE TO KEEP THE FRESH TISSUE SAMPLE (800-1000UL OF REAGENT FOR 80 TO 100 MG OF TISSUE).

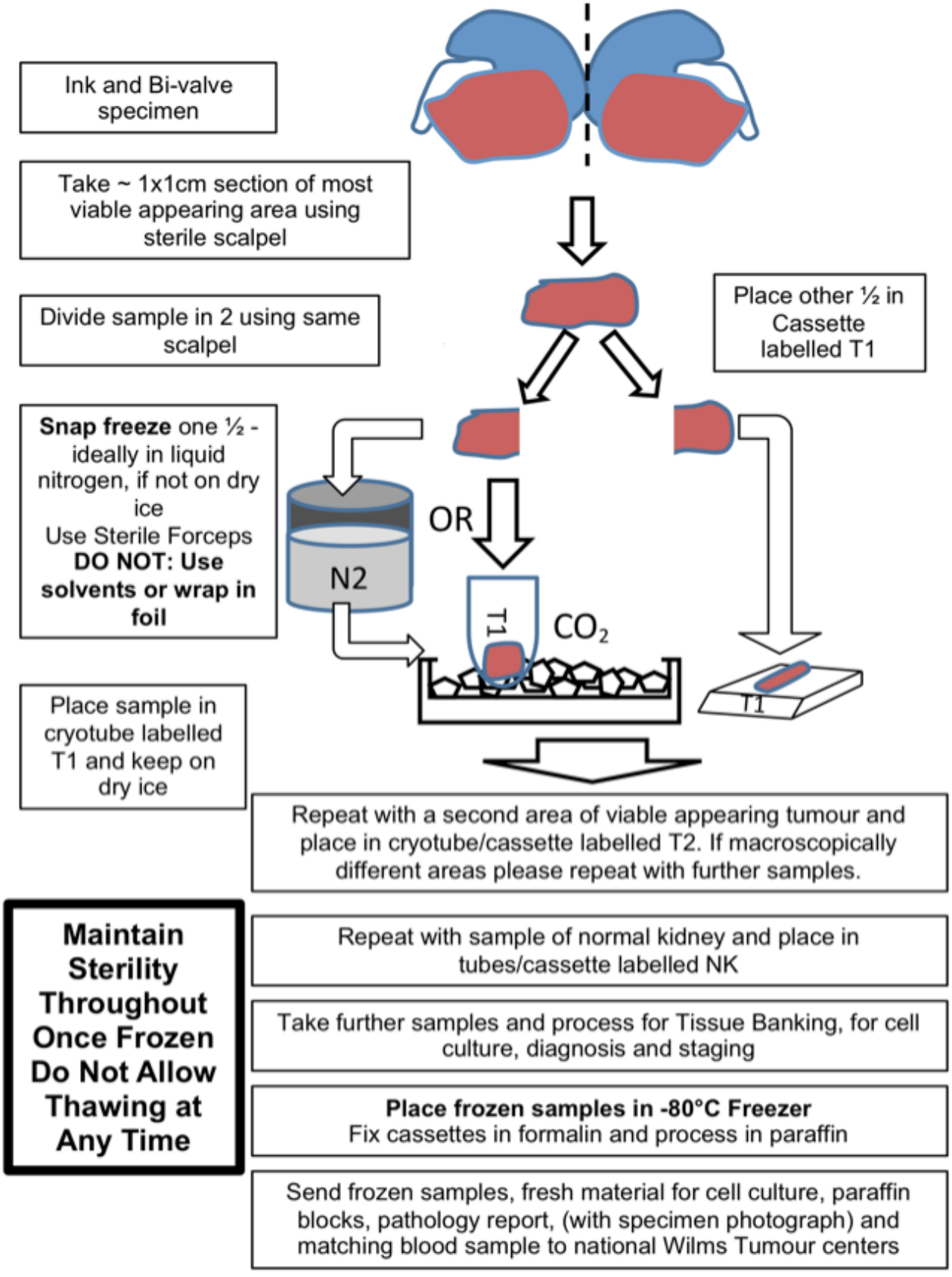

FIGURE 2. WORKFLOW FOR SUBMISSION OF TISSUE SAMPLES. SCHEME IS PART OF THE UMBRELLA SIOP–RTSG STUDY AND WAS KINDLY PROVIDED BY PROFESSOR KATHY PRITCHARD-JONES

one normal kidney samples from each case. All presented good quality for molecular analysis, pointing to the viability of this proposal.

These procedures are part of an attempt by the Brazilian Renal Tumors Group (BRTG) to promote research in renal tumors and to have active participation in molecular studies of international groups. Discussions regarding clinical, radiological, and pathological aspects of the patients are promptly discussed by email (tuwi_comite@googlegroups.com). More information is available at www.gbtr.com.br to increase participation in cooperative groups and improve diagnosis and treatment in such a heterogeneous country. All procedures were approved by the national ethical committee (CONEP 1.480.548; 2017).

Regarding WT pathology and molecular biology protocols, the BRTG complies with the UMBRELLA SIOP–RTSG 2016 updated guidelines, carefully reviewed by a consensus of pathology experts within the group, who also proposed to stimulate international collaboration with the harmonization of treatment protocols and research, including the standardization of specimen handling and improved collection of biological samples[35]. These are of utmost importance to validate biomarkers such as *MYCN* amplification, 1q gain, and 17p loss. After all, the development and inclusion of true predictive biomarkers for pediatric patients with WT will only be possible by enabling large, international, high-quality databases and samples within cooperative studies.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Author contributions

All authors made substantial contributions to the development of the study, providing samples, drafting the manuscript with critical revision, and providing final approval of the version to be published.

RESUMO

Os tumores renais pediátricos correspondem a aproximadamente 7% de todos os tumores infantis, sendo o mais frequente o tumor de Wilms (TW). Crianças com TW são geralmente tratadas seguindo dois distintos protocolos terapêuticos (COG ou SIOP), sendo que no último, os pacientes recebem tratamento quimioterápico pré-operatório. Ambos apresentam sobrevida global em cinco anos em torno de 90%. TW é geneticamente heterogêneo, apresentando baixa prevalência de alterações somáticas conhecidas, com cerca de 30% dos casos apresentando mutações em genes conhecidos e um alto grau de heterogeneidade genética intratumoral (HGIT). Além de potencialmente ter um impacto sobre o desfecho clínico dos pacientes, a HGIT pode interferir na busca de marcadores moleculares que estão sendo testados prospectivamente pelos grupos COG e Siop. Nesta revisão, apresentamos a proposta do atual estudo Umbrella Siop 2017/Grupo de Tumores Renais Brasileiros (GTRB), que orienta a coleta de três diferentes regiões do tumor para melhor avaliar possíveis marcadores moleculares, bem como para compreender a biologia do TW.

PALAVRAS-CHAVE: *Tumor de Wilms. Biomarcadores. Heterogeneidade genética.*

REFERENCES

1. Stiller CA, Parkin DM. International variations in the incidence of childhood renal tumours. Br J Cancer. 1990;62(6):1026-30.
2. Camargo B, Oliveira Ferreira JM, Souza Reis R, Ferman S, Oliveira Santos M, Pombo-de-Oliveira MS. Socioeconomic status and the incidence of non-central nervous system childhood embryonic tumours in Brazil. BMC Cancer. 2011;5;11:160.
3. Dumoucel S, Gauthier-Villars M, Stoppa-Lyonnet D, Parisot P, Brisse H, Philippe-Chomette P, et al. Malformations, genetic abnormalities, and Wilms tumor. Pediatr Blood Cancer. 2014;61(1):140-4.
4. Brok J, Treger TD, Gooskens SL, van den Heuvel-Eibrink MM, Pritchard-Jones K. Biology and treatment of renal tumours in childhood. Eur J Cancer. 2016;68:179-95.
5. D'Angio GJ. The National Wilms Tumor Study: a 40 year perspective. Lifetime Data Anal. 2007;13(4):463-70.
6. SIOP Renal Tumour Study Group. Paediatric renal tumours: perspectives from the SIOP-RTSG. Nat Rev Urol. 2017;14(1):3-4.
7. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler, KW. Cancer genome landscapes. Science. 2013;339(6127):1546-58.
8. Gadd S, Huff V, Walz AL, Ooms AHAG, Armstrong AE, Gerhard DS, et al. A Children's Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumor. Nat Genet. 2017;49(10):1487-94.
9. Mengelbier LH, Karlsson J, Lindgren D, Valind A, Lilljebjörn H, Jansson C, et al. Intratumoral genome diversity parallels progression and predicts outcome in pediatric cancer. Nat Commun. 2015;6:6125.

10. Scott RH, Murray A, Baskcomb L, Turnbull C, Loveday C, Al-Saadi R, et al. Stratification of Wilms tumor by genetic and epigenetic analysis. Oncotarget. 2012;3(3):327-35.

11. Ruteshouser EC, Robinson SM, Huff V. Wilms tumor genetics: mutations in WT1, WTX, and CTNNB1 account for only about one-third of tumors. Genes Chromosomes Cancer. 2008;47(6):461-70.

12. Torrezan GT, Ferreira EN, Nakahata AM, Barros BD, Castro MT, Correa BR, et al. Recurrent somatic mutation in DROSHA induces microRNA profile changes in Wilms tumour. Nat Commun. 2014;9;5:4039.

13. Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, et al. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. Cancer Cell. 2015;27(2):286-97.

14. Wegert J, Ishaque N, Vardapour R, Geörg C, Gu Z, Bieg M, et al. Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. Cancer Cell. 2015;27(2):298-311.

15. Rakheja D, Chen KS, Liu Y, Shukla AA, Schmid V, Chang TC, et al. Somatic mutations in DROSHA and DICER1 impair microRNA biogenesis through distinct mechanisms in Wilms tumours. Nat Commun. 2014;2:4802.

16. Maschietto M, Williams RD, Chagtai T, Popov SD, Sebire NJ, Vujanic G, et al. TP53 mutational status is a potential marker for risk stratification in Wilms tumour with diffuse anaplasia. PLoS One. 2014;9(10):e109924.

17. Williams RD, Chagtai T, Alcaide-German M, Apps J, Wegert J, Popov S, et al. Multiple mechanisms of MYCN dysregulation in Wilms tumour. Oncotarget. 2015;6(9):7232-43.

18. Segers H, van den Heuvel-Eibrink MM, Williams RD, van Tinteren H, Vujanic G, Pieters R, et al. Gain of 1q is a marker of poor prognosis in Wilms' tumors. Genes Chromosomes Cancer. 2013;52(11):1065-74.

19. Chagtai T, Zill C, Dainese L, Wegert J, Savola S, Popov S, et al. Gain of 1q as a prognostic biomarker in Wilms tumors (WTs) treated with preoperative chemotherapy in the International Society of Paediatric Oncology (SIOP) WT 2001 Trial: a SIOP Renal Tumours Biology Consortium Study. J Clin Oncol. 2016;34(26):3195-203.

20. Gratias EJ, Dome JS, Jennings LJ, Chi YY, Tian J, Anderson J, et al. Association of chromosome 1q gain with inferior survival in favorable-histology Wilms tumor: a report from the children's oncology group. J Clin Oncol. 2016;34(26):3189-94.

21. Grundy PE, Breslow NE, Li S, Perlman E, Beckwith JB, Ritchey ML, et al; National Wilms Tumor Study Group. Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National Wilms Tumor Study Group. J Clin Oncol. 2005;23(29):7312-21.

22. Spreafico F, Gamba B, Mariani L, Collini P, D'Angelo P, Pession A, et al. AIEOP Wilms Tumor Working Group. Loss of heterozygosity analysis at different chromosome regions in Wilms tumor confirms 1p allelic loss as a marker of worse prognosis: a study from the Italian Association of Pediatric Hematology and Oncology. J Urol. 2013;189(1):260-6.

23. Dix DB, Fernandez CV, Chi Y-Y, Anderson JR, Mullen EA, Geller JI, et al. Augmentation of therapy for favorable histology Wilms tumor combined with loss of heterozygosity of chromosomes 1p and 16q: a report from the Children's Oncology Group studies AREN0532 and AREN0533. J Clin Oncol. 2015;33:15.

24. Beckwith JB, Zuppan CE, Browning NG, Moksness J, Breslow NE. Histological analysis of aggressiveness and responsiveness in Wilms' tumor. Med Pediatr Oncol. 1996;27(5):422-8.

25. Maschietto M, Camargo B, Brentani H, Grundy P, Sredni ST, Torres C, et al. Molecular profiling of isolated histological components of Wilms tumor implicates a common role for the Wnt signaling pathway in kidney and tumor development. Oncology. 2008;75(1-2):81-91.

26. Charlton J, Williams RD, Sebire NJ, Popov S, Vujanic G, Chagtai T, et al. Comparative methylome analysis identifies new tumour subtypes and biomarkers for transformation of nephrogenic rests into Wilms tumour. Genome Med. 2015;7(1):11.

27. Fukuzawa R, Anaka MR, Morison IM, Reeve AE. The developmental programme for genesis of the entire kidney is recapitulated in Wilms tumour. PLoS One. 2017;12(10):e0186333.

28. Young MD, Mitchell TJ, Vieira Braga FA, Tran MGB, Stewart BJ, Ferdinand JR, et al. Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. Science. 2018;361(6402):594-9.

29. Perlman EJ. Pediatric renal tumors: practical updates for the pathologist. Pediatr Dev Pathol. 2005;8(3):320-38.

30. Rivera MN, Haber DA. Wilms' tumour: connecting tumorigenesis and organ development in the kidney. Nat Rev Cancer. 2005;5(9):699-712.

31. Cresswell GD, Apps JR, Chagtai T, Mifsud B, Bentley CC, Maschietto M, et al. Intra-tumor genetic heterogeneity in Wilms tumor: clonal evolution and clinical implications. EBioMedicine. 2016;9:120-9.

32. Wegert J, Wittmann S, Leuschner I, Geissinger E, Graf N, Gessler M. WTX inactivation is a frequent, but late event in Wilms tumors without apparent clinical impact. Genes Chromosomes Cancer. 2009;48(12):1102-11.

33. Spreafico F, Ciceri S, Gamba B, Torri F, Terenziani M, Collini P, et al. Chromosomal anomalies at 1q, 3, 16q, and mutations of SIX1 and DROSHA genes underlie Wilms tumor recurrences. Oncotarget. 2016;7(8):8908-15.

34. Wegert J, Vokuhl C, Ziegler B, Ernestus K, Leuschner I, Furtwängler R, et al. TP53 alterations in Wilms tumour represent progression events with strong intratumour heterogeneity that are closely linked but not limited to anaplasia. J Pathol Clin Res. 2017;3(4):234-48.

35. Vujanić GM, Gessler M, Ooms AHAG, Collini P, Coulomb-l'Hermine A, D'Hooghe E, et al; International Society of Paediatric Oncology–Renal Tumour Study Group (SIOP–RTSG). The UMBRELLA SIOP-RTSG 2016 Wilms tumour pathology and molecular biology protocol. Nat Rev Urol. 2018;15(11):693-701.

REVIEW ARTICLE

Pediatric tracheostomy: epidemiology and characterization of tracheal secretion - a literature review

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SUMMARY

INTRODUCTION: *Despite the benefits, tracheostomized children are susceptible to respiratory infections, since the tube is located in a strategic region where there is colonization by several bacteria and biofilm formation. Biofilm is formed when the bacteria adhere strongly to the surfaces of the tubes, providing protection against various types of aggression, such as antibiotic treatment.*

OBJECTIVE: *To carry out a literature review of the last ten years on tracheostomized pediatric patients, in order to characterize the bacteria isolated in children's tracheal secretions, and verify which ones are the most frequent.*

METHODS: *Two authors searched the Lilacs, SciELO, Medline Plus, and PubMed databases. The MeSH terms used were: 'tracheostomy' and 'tracheotomy' associated with 'infections', 'children', 'child', and 'bacterial' as qualifiers.*

RESULTS: *Of the 512 studies on the subject, 19 were selected for review. The total number of children evaluated in the studies was 4,472, with a mean age of 7.5 years. As for the bacteria found in the secretions of tracheostomized children, 12 species of bacteria were more frequent, P. aeruginosa was the predominant bacterium, followed by S. aureus (63.1%), Klebsiella pneumoniae (57.8%), Streptococcus pneumoniae (47.3%), and Stenotrophomonas maltophilia (47.3%).*

CONCLUSION: *One of the main complications treated in tracheostomized patients were infections, since the respiratory system is colonized by several bacteria that can cause serious infections, which are associated with the formation of biofilms. The predominant bacterium in most of the studies was P. aeruginosa, and the second species commonly reported was S. aureus.*

KEYWORDS: *Child. Trachea. Infection. Biofilms. Pseudomonas aeruginosa. Staphylococcus aureus.*

INTRODUCTION

Tracheostomy is a procedure that opens the airways by inserting a tube into the tracheal rings, allowing air to reach the lungs. The practice of tracheostomy has been reported since ancient times, for more than 2,000 years, and was described by the Egyptians through antique paintings[1]. Between the 1980s until mid-1990s, the indications for tracheostomy underwent great changes, since upper

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respiratory tract infections (URTI), such as diphtheria, were one of the most common reasons for performing the procedure. With the implementation of new immunization programs, *the epidemiological profiles of infections have changed, which reduced the need for the procedure*[2]. Currently, the indications for tracheostomy are acute respiratory failure, prolonged intubation, neurological disorders or lesions; the first two are the most common. Such indications increase the survival of children, especially of newborns, which results in an increased frequency of the procedure in pediatric patients[1,3].

Despite the benefits, children who undergo tracheostomy are more susceptible to respiratory infections. The presence of the tube diverts the air passage from the nasal and oral cavities, which provide natural protection, and creates a direct doorway for micro-organisms to enter the lower respiratory tract. The mucociliary clearance of the nasal mucosa and coughing, which clean the lower airways by expelling secretions and possible intruding agents, are also absent in these cases. Similarly, the long-term presence of the tube causes an inflammatory reaction of the tracheal mucosa, which increases the risk of infection since this is a strategic location for the colonization of several bacteria and for biofilm formation[4-6].

Biofilm is easily formed in this region because bacteria adhere strongly to the surfaces of tubes, forming a matrix that confers protection against various types of attacks, such as from the action of the immune system and antibiotics, which results in subsequent infections with greater frequency[5]. Bacterial pneumonia, aspiration pneumonia, and bacterial tracheitis were the pathologies reported with greater frequency in children who underwent tracheotomy, and bacterial pneumonia was for the highest number of hospitalizations[7].

The tracheostomy cannula is an environment with favorable conditions for the growth of *Pseudomonas aeruginosa (P. aeruginosa),* and a direct doorway, due to the incision of the trachea, for colonization by *Staphylococcus aureus (S. aureus)*. These species are found with greater frequency in tracheostomy tubes, but other micro-organisms have been reported too[2,4,5,8].

There is a clinical difficulty to identify the etiological agent involved in respiratory infections of tracheotomy patients, because tracheal secretion cultures also show colonizing bacteria, and are usually indicated when the child is already under an infectious process[6,9,10]. However, there is a need for greater caution in relation to the tracheal cultures in children who use a tracheotomy tube given that, in certain circumstances, they have some type of comorbidity or are hospitalized in Intensive Care Units, which makes them vulnerable to infections. In these cases, the culture and antibiogram are of extreme importance to guide appropriate treatment[1,2,4,6,11].

Due to the above, the objective of this study was to carry out a review of the literature of the past ten years on tracheotomy pediatric patients in order to characterize the bacteria found in tracheal aspirates.

METHODS

A literature review was conducted in the Latin America and Caribbean Health Sciences Literature (Lilacs), SciELO, National Library of Medicine (Medline Plus), and PubMed databases.

In the Medline and PubMed databases, the MeSH terms "tracheostomy" and "tracheotomy" were used associated with "infections", "children", "child", and "bacterial" as qualifiers.

On the SciELO, Lilacs, and Cochrane databases, we used combinations of the terms "tracheostomy" and "tracheotomy", "infections", "children", "child", and "bacterial".

The articles were evaluated independently by two of the authors of the study. The inclusion criteria for article selection were: English, Portuguese, or Spanish language, published over the past ten years (2008-2018), with participants aged from 0 to 15 years who used a tracheostomy tube, as well as studies that characterized the tracheal aspirates.

The exclusion criteria were: editorials, guidelines, advice, opinions, reviews, reports and case series, theses, as well as duplicate articles. We also excluded studies that did not evaluate the tracheal secretion, as well as studies on animals, studies of viral infections, studies with different types of samples, and studies that did not specify which samples were from tracheotomy patients.

RESULTS

During the research, we found 512 studies related to the subject, of which 19 were used for the review. The flowchart of the inclusion process is presented in Figure 1.

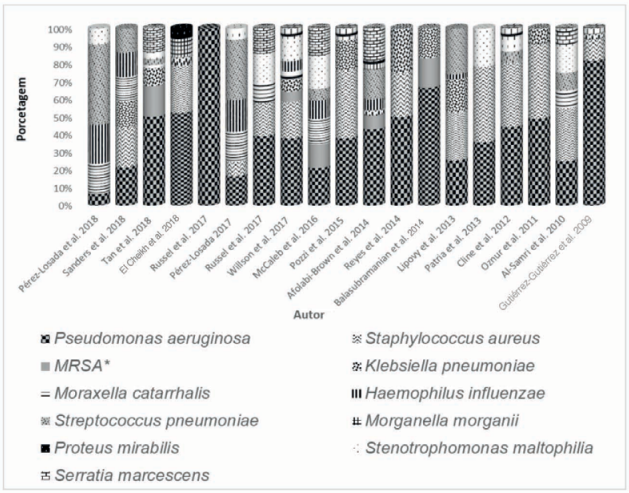
The total number of children evaluated in the studies selected was 4,472, with an average age of

7.5 years. The studies were conducted in 13 different countries, and the United States had the largest number of cases. Regarding gender, there was a 84% prevalence of males. With respect to the infection rates, 13 studies reported this index, of which two reported 100% of infection, six a rate higher than 50%, and the others a rate lower than 50% (Table 1).

All 19 studies described the bacteria found in the secretions of children who underwent tracheotomy. Twelve types of bacteria were more frequent and are described in Figure 3. *P. aeruginosa* was the most predominant bacteria, referenced in all studies, followed by *S. aureus* (63.1%), *Klebsiella pneumoniae* (57.8%), *Streptococcus pneumoniae* (47.3%), and *Stenotrophomonas maltophilia* (47.3%).

The bacteria found less frequently were *Proteus mirabilis* and *Morganella morganii*, mentioned in only two studies (Figure 2).

FIGURE 2. THE MAIN BACTERIA ISOLATED FROM TRACHEAL SECRETIONS OF CHILDREN WITH TRACHEOSTOMY TUBES REPORTED IN STUDIES ACCORDING TO THE AUTHORS.



DISCUSSION

We found in the present review several reports of tracheostomy in pediatric age patients, especially in children younger than 12 months[1,6,16,19], because the procedure is directly related to the increase of survival both of premature newborns with congenital malformations and of children of other ages who require mechanical ventilation for any particular purpose.

In relation to gender, from a total of 19 studies, 16 reported tracheotomy procedures with a greater frequency in male children [1,3,4,6-15,17-20]. This finding reflects the susceptibility of the gender to genetic or acquired diseases that require tracheostomy, which was also reported in another study on congenital diseases[9].

Several studies have cited the infections as the major complications in children who undergo

FIGURE 1. FLOWCHART OF STUDY SELECTION FOR LITERATURE REVIEW OF CHILDREN WHO UNDERWENT TRACHEOTOMY.

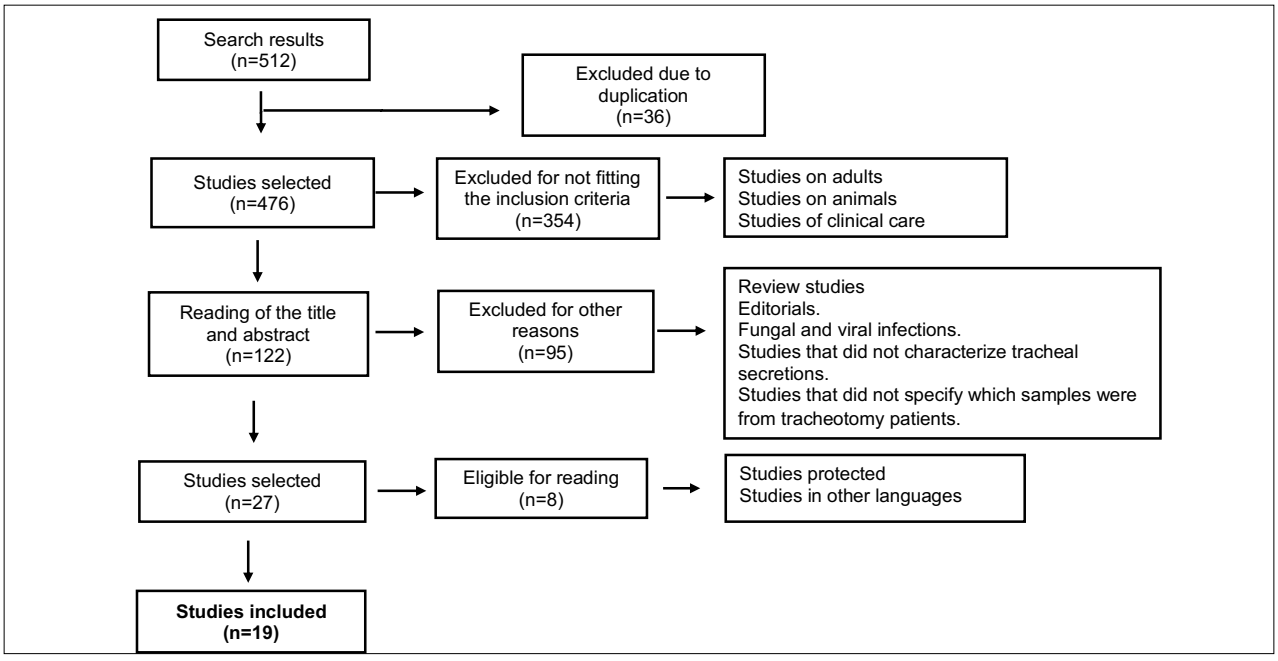


TABLE 1. CHARACTERISTICS OF THE STUDIES INCLUDED IN THE LITERATURE REVIEW OF CHILDREN WHO UNDERWENT TRACHEOTOMY.

Author	Country	Year of publication	n	Average age	Male	Infection
El Cheikh et al.[9]	Brasil	2018	20	2.8±3.6 y.	65%	NE
Pérez-Losada et al. [12]	Spain	2018	20	12 y.	70%	NE
Sanders et al. [5]	Colombia	2018	185	1.15 y.	NE	68%
Tan et al.[6]	China	2018	90	4.97 m.	65%	47%
Russell et al.[12]	USA	2017	3103	3 y.	57%	100%
Pérez-Losada et al.[3]	USA	2017	40	12.5 y.	73%	50%
Russell et al.[7]	USA	2017	103	NE	60%	100%
Willson et al. [13]	USA	2017	104	5.9 y.	78%	NE
McCaleb et al.[8]	USA	2016	93	0.84 y.	57%	71%
Pozzi et al. [14]	Italy	2015	65	NE	60%	51%
Afolabi-Brown et al.[15]	USA	2014	20	12.7±8.9 y.	65%	NE
Salcedo et al.[1]	Cuba	2014	14	2.5 y.	71%	71%
Balasubramanian & Tullu [16]	India	2014	19	9 m.	8%	NE
Lipový et al. [2]	Czech Republic	2013	31	1.7 y.	NE	NE
Patria et al. [17]	Italy	2013	115	4.5 y.	51%	NE
Cline et al.[4]	USA	2012	170	5.5 y.	61%	NE
Ak et al.[18]	Turkey	2011	83	NE	59%	26%
Al-Samri et al.[19]	United Arab Emirates	2010	72	3.4 m.	60%	90%
Gutiérrez-Gutiérrez et al.[20]	Costa Rica	2009	125	5.5 y.	66%	36%

m.: months. y.: years. USA: USA: United States of America. NE: Not evaluated.

tracheostomy, especially when there is prolonged use since the tube causes irritation of the trachea and facilitates bacterial colonization, predisposing the development of respiratory infections[1,5,19].

In the review of studies that characterized the bacteria in tracheal secretions, *P. aeruginosa* was the most prevalent[1-9,13-20]. Sanders et al.[5] highlight that children who use tracheotomy tubes for long periods are commonly colonized by *P. aeruginosa*, and in their study, there was an increase in the isolation of the bacteria after the use of tracheostomy. It is important to emphasize that the presence of the tube provides a direct link to the environment and that the protection mechanism of the upper respiratory tract is ineffective in such cases. In addition, *P. aeruginosa* is an environmental bacterium often found in hospital environments, and due to its great capacity to form biofilms, mainly in plastic devices such as tracheostomy tubes, its control is extremely important to prevent or avoid future complications[7]. It is worth noting that almost half of the studies reviewed (47.3%) were performed on children admitted to hospitals or Intensive Care Units (ICU) [1,2,4,6,7,14,16-18].

S. aureus was the second most frequently reported bacteria in in secretions from the trachea[1,2,4,5,9,11,13,14,17-20]. Despite being a bacteria that colonizes the respiratory tract and skin, it can become pathogenic and lead to serious infections, especially in patients with prolonged use of the tracheostomy tube[9]. *S. aureus* is associated with several diseases, mainly due to the ease of its transmission, as well as to the various mechanisms of resistance to antibiotics, such as resistance to methicillin and vancomycin. Its participation in infectious processes is also related to its ability to form biofilms, especially in chronic cases, which is an aggravating factor for therapeutic success[21].

The biofilm is formed with the adherence of bacteria to abiotic (plastics and metals) or biotic (tissue and cells) surfaces, developing a community surrounded by a polymeric extracellular matrix that confers protection against various types of attacks[22]. When we consider that the material of the studies reviewed is a biomedical device, implanted in a strategic region of the patient, such as the trachea, the reversible adhesion between bacteria and surface is favorable to the direct formation of a biofilm[23]. Such formation provides protection against the immune system response, antibiotics, lack of nutrients or water, among others[22]. Thus, the formation of biofilm represents a major

concern in the treatment and management of patients with a tracheotomy tube, mainly due to the difficulty of antibiotic action on these communities.

There are several mechanisms that hinder antimicrobial activity, since the presence of the polymeric matrix of the biofilm hinders the physical penetration of antibiotics. With this, there is a delay in their dissemination; bacteria in a biofilm present reduced metabolic and growth rates due to the nutrient limitation, which makes the action of medication more difficult, since most medications act when the bacteria are in the process of cell division[22] and the resistant bacteria present in a biofilm are able to degrade or inactivate antibiotics before they act in sensitive bacteria. In addition to these factors, phagocytes also have difficulty in destroying the micro-organisms because their entry into the matrix of the biofilm is extremely difficult[24]. All these factors show the vulnerability of antibiotics faced with a biofilm.

There is scarce information to guide professionals in the diagnosis and treatment of respiratory infections in children with tracheostomy tubes. It is known that the diagnosis can be achieved by X-ray of the thorax, but it can also be based on clinical criteria, such as increased tracheal secretions accompanied by fever with tachydyspnea. In addition, there is a difficulty in establishing the etiologic agent involved in respiratory diseases since cultures of tracheal secretions are indicated in severe cases, in which there is a need for hospitalization. It is known that among the pathogens of the respiratory system are, in addition to bacteria, viruses, and fungi. To detect specifically what etiological agent is causing the infection is a challenge and essential to implement the appropriate therapy[3,6,9,10,11].

Regarding the control of respiratory infections in patients with tracheostomy tubes, an important measure is to regularly change the cannula, which, according to the literature, should be done monthly in order to avoid the formation of biofilm, which, in turn, can lead to infections of the lower respiratory tract[10].

Given the relationship between tracheostomy and infections, more studies are needed on the subject to differentiate colonization from infection, and it is essential to standardize the prognosis, diagnosis, and appropriate therapy to control diseases associated with the tracheotomy patients.

CONCLUSION

Despite the numerous benefits of tracheostomy, studies show there are several complications related to it. The main complication addressed is infection. The predominant bacteria found in most studies was the *P. aeruginosa,* a species with a great capacity to cause respiratory infections, and its treatment can be hampered by its ability to form a biofilm. The second species frequently reported in tracheal secretions was *S. aureus*, which can also lead to respiratory complications due to the procedure.

There were other species of bacteria reported in tracheal secretions, such as *Klebsiella pneumoniae, Streptococcus pneumoniae,* and *Stenotrophomonas maltophilia.*

Contribution of the authors

All authors participated equally in the development of this work.

RESUMO

INTRODUÇÃO: *Apesar dos benefícios, crianças traqueostomizadas estão suscetíveis a adquirir infecções respiratórias, pois o tubo se encontra em uma região estratégica, na qual existe colonização de diversas bactérias e formação de biofilme. O biofilme é formado quando as bactérias aderem fortemente às superfícies dos tubos, conferindo proteção contra diversos tipos de agressões, como o tratamento por antibióticos.*

OBJETIVO: *Realizar uma revisão de literatura dos últimos dez anos sobre pacientes pediátricos traqueostomizados, no intuito de caracterizar as bactérias isoladas em secreções traqueais de crianças, verificando-se quais são as mais frequentes.*

MÉTODOS: *Dois autores pesquisaram nas bases de dados do Lilacs, SciELO, Medline Plus e PubMed. Termos MeSH utilizados: tracheostomy e tracheotomy usados associados a infections, children, chlid e bacterial como qualificadores.*

RESULTADOS: *Dos 512 estudos relacionados ao tema, 19 foram selecionados para a revisão. O total de crianças avaliadas nos estudos foi de 4.472, com idade média de 7,5 anos. Quanto às bactérias encontradas nas secreções de crianças traqueostomizadas, 12 espécies de bactérias foram mais frequentes; P. aeruginosa foi a bactéria predominante, seguida de S. aureus (63,1%), Klebsiella pneumoniae (57,8%), Streptococcus pneumoniae (47,3%) e Stenotrophomonas maltophilia (47,3%).*

CONCLUSÃO: *Umas das principais complicações abordadas em pacientes traqueostomizados foram as infecções, já que o sistema respiratório é colonizado por diversas bactérias, que podem causar infecções graves, sendo estas associadas à formação de biofilmes. A bactéria predominante na maioria dos estudos foi a P. aeruginosa, e a segunda espécie comumente relatada foi a S. aureus.*

PALAVRAS-CHAVE: *Criança. Traqueia. Infecção. Biofilmes. Pseudomonas aeruginosa. Staphylococcus aureus.*

REFERENCES

1. Salcedo C, Martínez M, Reyes E. Pediatric tracheostomy: a ten-year analysis in the Intensive Care Unit of Sancti Spiritus "José Martí" Pediatric Teaching Hospital. Medwave. 2014;14(4):e5949.
2. Lipový B, Brychta P, Řihová H, Suchanek I, Hanslianová M, Cvanová M, et al. Effect of timing of tracheostomy on changes in bacterial colonisation of the lower respiratory tract in burned children. Burns. 2013;39(2):255-61.
3. Pérez-Losada M, Graham RJ, Coquillette M, Jafarey A, Castro-Nallar E, Aira M, et al. The temporal dynamics of the tracheal microbiome in tracheostomised patients with and without lower respiratory infections. PLoS One. 2017;12(8):e0182520.
4. Cline JM, Woods CR, Ervin SE, Rubin BK, Kirse DJ. Surveillance tracheal aspirate cultures do not reliably predict bacteria cultured at the time of an acute respiratory infection in children with tracheostomy tubes. Chest. 2012;141(3):625-31.
5. Sanders CD, Guimbellot JS, Muhlebach MS, Lin FC, Gilligan P, Esther CR Jr. Tracheostomy in children: epidemiology and clinical outcomes. Pediatr Pulmonol. 2018;53(9):1269-75.
6. Tan CY, Chiu NC, Lee KS, Chi H, Huang FY, Huang DT, et al. Respiratory tract infections in children with tracheostomy. J Microbiol Immunol Infect. 2018. pii: S1684-1182(18)30284-6.
7. Russell CJ, Simon TD, Mamey MR, Newth CJL, Neely MN. Pseudomonas aeruginosa and post-tracheotomy bacterial respiratory tract infection readmissions. Pediatr Pulmonol. 2017;52(9):1212-8.
8. McCaleb R, Warren RH, Willis D, Maples HD, Bai S, O'Brien CE. Description of respiratory microbiology of children with long-term tracheostomies. Respir Care. 2016;61(4):447-52.
9. El Cheikh MR, Barbosa JM, Caixêta JAS, Avelino MAG. Microbiology of tracheal secretions: what to expect with children and adolescents with tracheostomies. Int Arch Otorhinolaryngol. 2018;22(1):50-4.
10. Avelino MAG, Maunsell R, Valera FCP, Lubianca Neto JF, Schweiger C, Miura CS, et al. First Clinical Consensus and National Recommendations on Tracheostomized Children of the Brazilian Academy of Pediatric Otorhinolaryngology (ABOPe) and Brazilian Society of Pediatrics (SBP). Braz J Otorhinolaryngol. 2017;83(5):498-506.
11. Russell CJ, Mack WJ, Schrager SM, Wu S. Care variations and outcomes for children hospitalized with bacterial tracheostomy-associated respiratory infections. Hosp Pediatr. 2017;7(1):16-23.
12. Pérez-Losada M, Graham RJ, Coquillette M, Jafarey A, Castro-Nallar E, Aira M, et al. Tracheal microbiota in patients with a tracheostomy before, during, and after an acute respiratory infection. Pediatr Infect Dis J. 2018;37(11):e269-71.
13. Willson DF, Hoot M, Khemani R, Carrol C, Kirby A, Schwarz A, et al; Ventilator-Associated INfection (VAIN) Investigators and the Pediatric Acute Lung Injury and Sepsis Investigator's (PALISI) Network. Pediatric ventilator-associated infections: the ventilator-associated infection study. Pediatr Crit Care Med. 2017;18(1):e24-e34.
14. Pozzi M, Pellegrino P, Galbiati S, Granziera M, Locatelli F, Carnovale C, et al. Prevalence of respiratory colonisations and related antibiotic resistances among paediatric tracheostomised patients of a long-term rehabilitation centre in Italy. Eur J Clin Microbiol Infect Dis. 2015;34(1):169-75.
15. Afolabi-Brown O, Marcus M, Speciale P, Pagala M, Kazachkov M. Bronchoscopic and nonbronchoscopic methods of airway culturing in tracheostomized children. Respir Care. 2014;59(4):582-7.
16. Balasubramanian P, Tullu MS. Study of ventilator-associated pneumonia in a pediatric intensive care unit. Indian J Pediatr. 2014;81(11):1182-6.
17. Patria MF, Chidini G, Ughi L, Montani C, Prandi E, Galeone C, et al. Ventilator-associated pneumonia in an Italian pediatric intensive care unit: a prospective study. World J Pediatr. 2013;9(4):365-8.
18. Ak O, Batirel A, Ozer S, Çolakoğlu S. Nosocomial infections and risk factors in the intensive care unit of a teaching and research hospital: a prospective cohort study. Med Sci Monit. 2011;17(5):PH29-34.
19. Al-Samri M, Mitchell I, Drummond DS, Bjornson C. Tracheostomy in children: a population-based experience over 17 years. Pediatr Pulmonol. 2010;45(5):487-93.
20. Gutiérrez-Gutiérrez I, Solano-Blanco FP, Gutiérrez-Schwanhauser JB. Original experiencia de la clínica de traqueostomía del Hospital Nacional de Niños "Dr. Carlos Sáenz Herrera". Acta Med Costarric. 2009;51(4):215-21.
21. Bhattacharya M, Wozniak DJ, Stoodley P, Hall-Stoodley L. Prevention and treatment of *Staphylococcus aureus* biofilms. Expert Rev Anti Infect Ther. 2015;13(12):1499-516.
22. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms : a common cause of persistent infections. Science. 1999;284(5418):1318-22.
23. Dunne WM Jr. Bacterial adhesion : seen any good biofilms lately? Clin Microbiol Rev. 2002;15(2):155-66.
24. Simões M, Simões LC, Vieira MJ. Species association increases biofilm resistance to chemical and mechanical treatments. Water Res. 2009;43(1):229-37.

REVIEW ARTICLE

Low-dose CT screening can reduce cancer mortality: A meta-analysis

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SUMMARY

OBJECTIVE: *Lung cancer is the leading cause of cancer-related death. To reduce lung cancer mortality and detect lung cancer in early stages, low dose CT screening is required. A meta-analysis was conducted to verify whether screening could reduce lung cancer mortality and to determine the optimal screening program.*

METHODS: *We searched PubMed, Web of Science, Cochrane library, ScienceDirect, and relevant Chinese databases. Randomized controlled trial studies with participants that were smokers older than 49 years (smoking >15 years or quit smoking 10 or 15 years ago) were included.*

RESULTS: *Nine RCT studies met the criteria. LDCT screening could find more lung cancer cases (RR=1.58, 95%CI=1.25-1.99, P<0.001) and more stage I lung cancers (RR=3.45, 95%CI=2.08-5.72, P<0.001) compared to chest-X ray or the no screening group. This indicated a statistically significant reduction in lung-cancer-specific mortality (RR=0.84, 95%CI=0.75-0.95, P=0.004), but without a statistically reduction in mortality due to all causes (RR=1.26, 95%CI=0.89-1.78, P=0.193). Annually, LDCT screening was sensitive in finding more lung cancers.*

CONCLUSIONS: *Low-dose CT screening is effective in finding more lung cancer cases and decreasing the deaths from lung cancer. Annual low-dose CT screening may be better than a biennial screening to detect more early-stage lung cancer cases.*

KEYWORDS: *Randomized controlled trial. Meta-analysis. Triage. Early Detection of Cancer. Tomography, X-Ray Computed.*

INTRODUCTION

Lung cancer is among the most frequently diagnosed cancer types and is the leading cause of cancer deaths, with 1.82 million new cases and 1.6 million deaths in 2012 [1,2]. Lung cancer has a poor prognosis and a relatively low five-year relative survival ratio in relation to all types of cancer; 5-year survival in Europe ranges from 9.6% in the UK to 17.9% in Austria. Lung cancer patients have poor outcomes of treatment, resulting from the fact that most cases are diagnosed in the advanced stage of the disease. Nowadays, some studies, like the NLST, advocate that screening for lung cancer in specific high-risk

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groups of smokers could reduce mortality[3]. The EU Position Statement (EUPS) argues that lung cancer screening with LDCT could save lives, and planning for implementation throughout Europe has started[4]. A meta-analysis containing nine randomized controlled trials and review articles up to 2013 showed that LDCT screening for lung cancer resulted in the detection of a greater total of lung cancer cases, stage I lung cancers, and decreased lung cancer-specific mortality[5].

Apart from verifying whether LDCT screening could reduce mortality due to lung cancer in high-risk smokers, we also explored whether a less frequent screening scenario, like biennial screening, could be equally effective as the annual screening? UPS also considered the biennial screening scenario before [6].

METHODS

Data source and search strategy

Published studies were extensively searched through PubMed, Web of Science, Cochrane library, ScienceDirect (SD), CNKI (China National Knowledge Infrastructure), and CBM (Chinese biomedical Database) in order to seek RCTs related to LDCT or X-ray screening for lung cancer. The date of the last search was February 26, 2019. To obtain additional relevant studies, we also scanned conference proceedings and reference lists. The following search terms were used: "LDCT", "low dose helical CT", "low dose CT", "Low-dose spiral CT", "Low dose computed tomography", "lung neoplasm", "lung tumor", "lung carcinoma", "lung cancer".

Inclusion Criteria and Exclusion Criteria

Each potentially eligible article was checked to see if they met all the following inclusion criteria: (1) the study design was a RCT (randomized controlled trial) comparing LDCT with x-ray or usual care; (2)participants were smokers who had been smoking >15 years or former smokers who stopped smoking 10 or 15 years ago; (3) participants were over 49 years old; (4) the data of all lung cancers, stage I lung cancers, lung cancer-specific mortality, all-cause mortality were reported in detail; (5) for duplicated publications on the same population, the most recent ones with the most complete data set were included. The exclusion criteria were as follows: (1) the screening only involved other risk factors, like asbestos; (2) the participants had a history of previous cancer with a clinically established diagnosis.

Data extraction and quality assessment

Two investigators independently extracted the following data for each eligible study: name of the study, first author, year of publication, sample size, trial randomization, age, sex, smoking history, screening in past years, screening times, screen interval, total follow-up time. If there was any dispute, it would be solved by a third independent reviewer. The NHS Critical Appraisal Skills Programme (CASP) critical appraisal tool for RCTs was chosen for this meta-analysis as it covers sequence generation, allocation sequence concealment, blinding, incomplete outcome data, and selective outcome reporting [7].

Statistical Method

The relative risk (RR), 95% confidence interval (CI), and Forest Plot were produced using STATA14.0 to measure the effect of the low-dose CT screening on lung cancer. We calculated the Q-statistic (χ^2) and I^2 statistics to quantify the heterogeneity. Heterogeneity was classified as low, moderate, and high when the cut-off points of I^2 values reached 25%, 50%, or 75% respectively[8]. If I^2>50% (I^2-statistic) or P<0.1 (Q-statistic), it indicated an obvious heterogeneity; then, the random-effect model (DerSimonian and Laid method)[9] would be used. Otherwise, the fixed-effects model (Mantel–Haenszel method)[10] was used. Furthermore, subgroup analyses were conducted to explore the source of heterogeneity. The potential publication bias was assessed by using Begg's test[11] and Egger's Test [12], which might exist when p< 0.05. Sensitivity analysis was used to search the extent to which inferences might depend on a particular study or group of studies. Differences were considered as statistically significant if two-sided P-values were less than 0.05.

RESULTS

Characteristics of the eligible studies

In order to identify all eligible studies, a comprehensive process was performed. A total of 1434 articles were collected and reviewed. After excluding duplicated papers, 1167 studies were left. Of these, 1107 were removed since they were found to be review studies or not on the topic after screening the title and/or abstract. The remaining 60 full-text articles were assessed for eligibility. Eventually, 9 eligible articles were included in the final meta-analysis (Figure 1), and their details are presented in table 1 [3,13-20]. Their quality ranged from moderate to high; most studies

TABLE1. CHARACTERISTICS OF THE STUDIES INCLUDED IN THIS META-ANALYSIS.

Name of the study	Sample Size (study: control)	Control	Age	Male (%)	Smoking History (py)/Former Smokers (yr)	Screening interval (year)	Screen-ing times	Median screening years	Median Follow-up years
NLST[3,29] 2011	53454 (1:1)	CXR	55-74	59.0	>30/<15	1	3	5	6.5
Danish[20] 2015	4104 (1:1)	usual care	50-70	50.0	>20/<10	1	5	4.81	9.8*
Dante[17] 2015	2450 (1:1)	CXR	60-74	100.0	>20/<10	1	5	7	8.35
MILD[13] 2012	4099 (2:2:3)	usual care	>65	66.3	>20/<10	1/2	5/3	4.4	4.4
German[19] 2015	4052 (1:1)	usual care	50-69	64.7	>15/<10	1	3	5	5
Depiscan[14] 2007	765 (1:1)	CXR	50-75	70.7	>20/<15	1	1	2	2
LSS[16] 2004	3318 (1:1)	CXR	55-74	59.0	>30/<10	1	2	2	2
ITALUNG[18] 2017	3206 (1:1)	usual care	55-69	65.2	>20/<10	1	4	9.3	9.3
China[15] 2018	472 (1:1)	usual care	55-74	-	>30/<15	2	3	2	2

py =pack-year *9.8person-y. The sex ratio in China was not displayed in the NLST criteria population concretely, but males were the majority.

FIGURE 1.

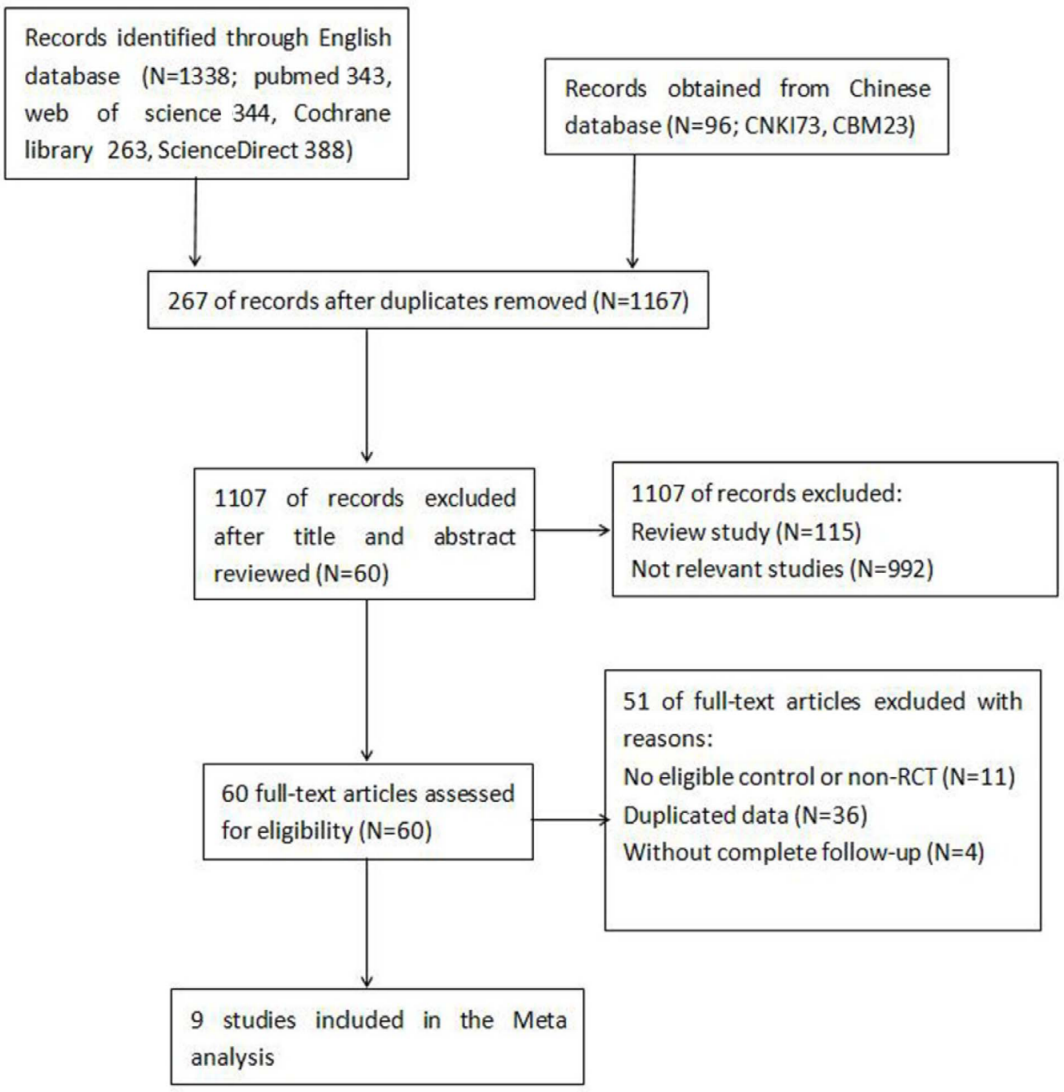
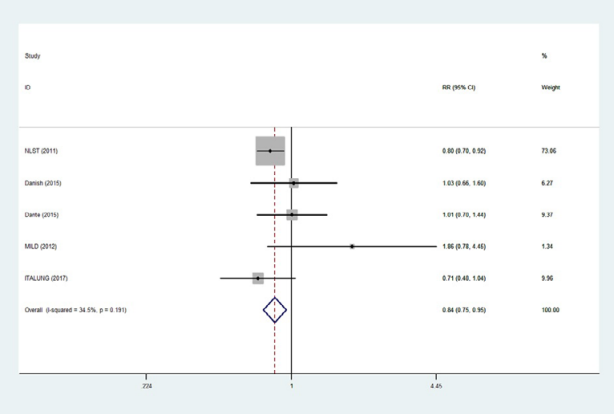


FIGURE 2.



had explicit random assignment, concealed allocation, follow-up details, definite diagnosing criterion, and CT protocol. However, the double-blind could not be satisfied in the screening since the screening methods were evidently different between the treatment and control groups.

The nine studies included 38357 LDCT individuals and 37563 controls, both from high-risk lung cancer populations. The range of age was 50 to 70 years old; the average smoking history was 20 years, and the total screening years varied from 2 to 9.8 years. There were more males than females included in this analysis since most heavy smokers are male. The participants included in the meta-analysis were from Denmark, Italy, Germany, France, UK, China, and the United States.

Detection rate of total lung cancers

LDCT could find more lung cancers than the CXR or the no-screening group (RR=1.58, 95%CI=1.25-1.99, *P*<0.001), with high heterogeneity (χ^2=32.96, I^2=72.7%, *P*<0.001). In the meta-analysis pooled result, we could find more lung cancers by screening no matter how long the total follow-up was, i.e., more than 5 years (RR=1.35, 95%CI=1.06-1.72, *P*=0.015) or less than 5 years (RR=2.16, 95%CI=1.59-2.95, *P*<0.001). The annual screening (RR=1.67, 95%CI=1.25-2.24, *P*<0.001) had a statistical significance in finding more lung cancers than biennial screening (RR=1.35, 95%CI=0.91-2.00, *P*=0.140).

Detection rate of stage I lung cancers

The pooled data of stage I lung cancers indicated that more early-stage cancers can be found by LDCT screening (RR=3.45, 95%CI=2.08-5.72, *P*<0.001), with high Heterogeneity (χ^2=25.74, I^2=72.8%, *P*=0.001). The low dose CT, with absolute superiority, could find not only more lung cancers but also more stage I lung cancers.

After we proceeded to subgroup analyses regarding the detection rate of stage I lung cancers between the different types in the control group, we found there was no significant Heterogeneity in the CXR screening population [I^2=0.0%, *P*=0.463].

Lung Cancer-Specific Mortality and All-cause mortality

Five studies reported Lung-Cancer-Specific Mortality. After merging, we found the protective effect in the LDCT group was statistically significant compared to the control group (RR=0.84, 95%CI=0.75-0.95, *P*=0.004), with moderate Heterogeneity (χ^2=6.11, I^2=34.5%, *P*=0.191). The merging result is presented in Fig 2. Six studies provided information on all-cause mortality. The results showed that there was no statistically significant difference between the two groups (RR=1.26, 95%CI=0.89-1.78, *P*=0.193), with high Heterogeneity in the data (χ^2=99.08, I^2=95.0%, *P*<0.001).

Sensitivity Analysis and Publication bias evaluation

To assess the stability of the studies, we performed a sensitivity analysis using the leave-one-out method and reviewed the consistency of the results. We discarded each individual study to recalculate the Relative Risk. However, no single study influenced the pooled results significantly. Sensitivity analysis showed that the overall effect was evenly distributed in the trials included. The Begg's Test and Egger's Test were performed to evaluate publication bias of all the 9 studies. The results of Begg's Test (Begg's test: P=0.161) showed that the potential publication bias had no obvious influence, but Egger's Test indicated publication bias (Eegg's test: *P*< 0.001).

DISCUSSION

In this analysis, we calculated both the detection rate and mortality. The results demonstrated that the low-dose spiral CT had an absolute advantage compared to the CXR in lung cancer screening to find a higher proportion of lung cancers and stage-I cancers. Besides, it can provide a reduction of lung cancer-specific mortality in the low dose CT screening

group. As to deaths due to all causes, it did not manifest a protective effect; this may be because smoking is a risk factor of many cardiovascular diseases, which were the cause of death for older smokers. Moreover, mortality for causes other than lung cancer revealed that screening has no effect in reducing these deaths. Our results are consistent with the meta-analysis by Cuiping FU et al.[5] regarding reduced lung-cancer-specific mortality. Compared to the meta-analysis by Cuiping FU et al. who, after collecting information in 2013, published, in 2016, two RCT articles not included in this meta-analysis, the American feasibility study [21] and the NELSON trial, [22] since they did not have complete two-arm follow-up, nor did they report the results of the control group. The UK Lung Cancer Screenings in 2016 have not reported the results of the control group either [23,24]. To make the pooled results more reliable, the three RCT articles were not included in this analysis. Besides, the results did not vary greatly when adding the three studies in the meta-analysis. Furthermore, the lung cancer screening results of Danish, Dante, and Italung were updated in this analysis. Likewise, one 2015 study from German and one from China in 2018 were added in this analysis.

Then we considered the potential optimal interval of screening. The biennial screening has lower heterogeneity than the annual LDCT screening by subgroup analysis. Annual screening could find more lung cancers than chest-X rays or the no screening group, and the difference was statistically significant. However, the difference in lung cancer detection rate between the biennial screening and chest-X rays or the no screening group were not statistically significant. We also discussed the NELSON trial, in which the screening arm received LDCT screening at baseline, after 1 , 2, and 2.5 years [22]. No significant differences were found in the detection rates between the 1-year and the 2-year interval screening, but stage distributions were different. Compared with 1-year interval screening, a lower proportion of stage I and a higher proportion of advanced (stage IIIb/IV) cancers were detected in the 2.5-year interval round; this difference was statistically significant. The 2.5-year round showed no statistical significance in stage distributions compared with the 2-year round. This indicates that the 2.5 years interval is too long, which reduced the effect of the screening. However, in the MILD trial, there was no statistical difference in stage distribution or mortality between the annual and biennial CT screening [13]. Therefore, more qualified studies are needed to demonstrate the optimal interval of lung cancer screening.

Although lung cancer screening could detect more new lung cancers, its benefits and harms must be considered, such as the high rate of false positives and its cost-effectiveness, before widespread low-dose CT screening is implemented. In 2017, the European Union position statement declared that Lung cancer screening with low-dose CT could save lives, and it should start being implemented throughout Europe as soon as possible[4].

Limitations of our study include the problem of the heterogeneity, which generally exists in many meta-analyses. What is more, a few articles did not have important data, which could not be obtained even after we wrote to the authors. Furthermore, the publication bias requires us to do more research with a higher-quality and larger sample. Furthermore, we only pooled the results of articles on smokers, but will other populations at risk of lung cancer benefit from CT screening? We could not draw a conclusion limited to finite studies. Some studies reported that screening might have an unintended health certificate effect in changing their lifestyle[25] and cause them to give up or reduce smoking habits[26]. This indicates that lung cancer screening should be accompanied by a smoking cessation intervention in widespread populations to reduce lung cancer mortality since smoking cessation is the best and the most cost-effective approach to reducing the risk of lung cancer[27]. Furthermore, the Dutch-Belgian Lung Cancer Screening trial (NELSON) is an ongoing randomized controlled trial that evaluates LDCT with a target of 10-year follow up [22]. NELSON will assess survival, quality of life, smoking cessation, and cost-effectiveness. We are waiting for the results.

CONCLUSION

In conclusion, LDCT screening for lung cancer has been demonstrated to reduce deaths from lung cancer in high-risk smokers. Given its high sensibility to find early-stage lung cancer, it is promising to identify and cure more lung cancers in the early stage. An annual LDCT screening is effective in finding more early-stage lung cancers. More studies on both the positive and negative aspects are required to enrich the conclusion and make further efforts to reduce morbidity and mortality from lung cancer.

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Author contributions

Xue Tang and Wei Wu conceived the study. Xue Tang, Lingling Wang, and Guangbo Qu oversaw data collection. Xue Tang conducted the analysis. Xue Tang drafted the manuscript, and all authors contributed to its critical review. Xue Tang assumes overall responsibility for the paper. The authors thank colleagues at the Anhui Medical University, BAO DONG for the help with methodology support, and YAN-JIE ZHANG for support in the literature search.

RESUMO

OBJETIVO: *O câncer de pulmão é a principal causa de mortes relacionadas ao câncer. Para reduzir a mortalidade por câncer de pulmão e encontrar câncer de pulmão em um estágio inicial, é necessária uma triagem por tomografia de baixa dose. Uma meta-análise foi emitida para testemunhar se a triagem poderia reduzir a mortalidade por câncer de pulmão e investigar o melhor programa de triagem.*

MÉTODOS: *Pesquisamos PubMed, Web of Science, biblioteca Cochrane, ScienceDirect e relevante banco de dados chinês. Ensaios clínicos controlados aleatórios, em que os participantes eram fumantes com mais de 49 anos (tabagismo >15 anos ou parar de fumar 10 ou 15 anos atrás) foram incluídos.*

RESULTADOS: *Nove estudos RCT preencheram os critérios. O rastreamento de LDCT pôde encontrar mais cânceres de pulmão (RR=1,58, IC 95%=1,25-1,99, P<0,001) e mais cânceres de estágio I do pulmão (RR=3,45, IC 95%=2,08-5,72, P<0,001) em comparação com raio X do tórax ou nenhum grupo de triagem. Ele indicou uma redução estatisticamente significativa na mortalidade específica do câncer de pulmão (RR=0,84, IC 95%=0,75-0,95, P=0,004), mas sem uma redução estatisticamente significativa na mortalidade por todas as causas (RR=1,26, IC 95%=0,89-1,78, P=0,193). Anualmente, o rastreamento de LDCT foi sensível em encontrar mais cânceres de pulmão.*

CONCLUSÕES: *A triagem de TC de baixa dose é eficaz para encontrar mais cânceres de pulmão e diminuir as mortes por câncer de pulmão. Para encontrar mais cânceres de pulmão em estágio inicial, a triagem anual de tomografia de baixa dose pode ser melhor do que a triagem bianual.*

PALAVRAS-CHAVE: *Ensaio clínico controlado aleatório. Meta-análise. Triagem. Detecção precoce de câncer. Tomografia computadorizada por raios X.*

REFERENCES

1. Didkowska J, Wojciechowska U, Mańczuk M, Lobaszewski J. Lung cancer epidemiology: contemporary and future challenges worldwide. Ann Transl Med. 2016;4(8):150.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
3. National Lung Screening Trial Research Team, Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD, Fagerstrom RM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med. 2011;365(5):395-409.
4. Oudkerk M, Devaraj A, Vliegenthart R, Henzler T, Prosch H, Heussel CP, et al. European position statement on lung cancer screening. Lancet Oncol. 2017;18(12):e754-66.
5. Fu C, Liu Z, Zhu F, Li S, Jiang L. A meta-analysis: is low-dose computed tomography a superior method for risky lung cancers screening population? Clin Respir J. 2016;10(3):333-41.
6. Field JK, Baldwin DR, Devaraj A, Oudkerk M. EUPS-argues that lung cancer screening should be implemented in 18 months. Br J Radiol. 2018;91(1090):20180243.
7. Critical Appraisal Skills Programme. 11 questions to help you make sense of a trial, 2013. [cited 2019 Feb 5]. Available from: http://www.casp-uk.net/casp-tools-checklists
8. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.
9. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177-88.
10. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959;22(4):719-48.
11. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088-101.
12. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-34.
13. Pastorino U, Rossi M, Rosato V, Marchiano A, Sverzellati N, Morosi C, et al. Annual or biennial CT screening versus observation in heavy smokers: 5-year results of the MILD trial. Eur J Cancer Prev. 2012;21(3):308-15.
14. Blanchon T, Bréchot JM, Grenier PA, Ferretti GR, Lemarié E, Milleron B, et al; Dépiscan Group. Baseline results of the Depiscan study: a French randomized pilot trial of lung cancer screening comparing low dose CT scan (LDCT) and chest X-ray (CXR). Lung Cancer. 2007;58(1):50-8.
15. Yang W, Qian F, Teng J, Wang H, Manegold C, Pilz LR, et al. Community-based lung cancer screening with low-dose CT in China: results of the baseline screening. Lung Cancer. 2018;117:20-6.
16. Gohagan JK, Marcus PM, Fagerstrom RM, Pinsky PF, Kramer BS, Prorok PC, et al. Final results of the Lung Screening Study, a randomized feasibility study of spiral CT versus chest X-ray screening for lung cancer. Lung Cancer. 2005;47(1):9-15.
17. Infante M, Cavuto S, Lutman FR, Passera E, Chiarenza M, Chiesa G, et al; DANTE Study Group. Long-term follow-up results of the DANTE trial, a randomized study of lung cancer screening with spiral computed tomography. Am J Respir Crit Care Med. 2015;191(10):1166-75.

18. Paci E, Puliti D, Lopes Pegna A, Carrozzi L, Picozzi G, Falaschi F, et al. Mortality, survival and incidence rates in the ITALUNG randomised lung cancer screening trial. Thorax. 2017;72(9):825-31.

19. Becker N, Motsch E, Gross ML, Eigentopf A, Heussel CP, Dienemann H, et al. Randomized study on early detection of lung cancer with MSCT in Germany: results of the first 3 years of follow-up after randomization. J Thorac Oncol. 2015;10(6):890-6.

20. Wille M, Dirksen A, Ashraf H, Saghir Z, Bach KS, Brodersen J, et al. Results of the randomized Danish lung cancer screening trial with focus on high-risk profiling. Am J Respir Crit Care Med. 2016;193(5):542-51.

21. Garg K, Keith RL, Byers T, Kelly K, Kerzner AL, Lynch DA, et al. Randomized controlled trial with low-dose spiral CT for lung cancer screening: feasibility study and preliminary results. Radiology. 2002;225(2):506-10.

22. Yousaf-Khan U, van der Aalst C, Jong PA, Heuvelmans M, Scholten E, Lammers JW, et al. Final screening round of the NELSON lung cancer screening trial: the effect of a 2.5-year screening interval. Thorax. 2017;72(1):48-56.

23. Field JK, Duffy SW, Baldwin DR, Brain KE, Devaraj A, Eisen T, et al. The UK Lung Cancer Screening Trial: a pilot randomised controlled trial of low-dose computed tomography screening for the early detection of lung cancer. Health Technol Assess. 2016;20(40):1-146.

24. Baldwin DR, Duffy SW, Wald NJ, Page R, Hansell DM, Field JK. UK Lung Screen (UKLS) nodule management protocol: modelling of a single screen randomised controlled trial of low-dose CT screening for lung cancer. Thorax. 2011;66(4):308-13.

25. van der Aalst CM, van Klaveren RJ, Koning HJ. Does participation to screening unintentionally influence lifestyle behaviour and thus lifestyle-related morbidity? Best Pract Res Clin Gastroenterol. 2010;24(4):465-78.

26. Filippo L, Principe R, Cesario A, Apolone G, Carleo F, Ialongo P, et al. Smoking cessation intervention within the framework of a lung cancer screening program: preliminary results and clinical perspectives from the "Cosmos-II" Trial. Lung. 2015;193(1):147-9.

27. Tota JE, Ramanakumar AV, Franco EL. Lung cancer screening: review and performance comparison under different risk scenarios. Lung. 2014;192(1):55-63.