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- Aberrant antigens in acute leukemia outcome

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Cover: Confocal image of the epidermis from a patient with melanoma, showing the expression pattern of α-synuclein and tyrosinase. Tyrosinase expression (blue) indicates the location of the melanocytes in the stratum basale; the enzyme is predominantly located in the cytoplasm. By contrast, α-synuclein (labeled with Alexa Fluor 488, green color) is found in the nucleus and the cytoplasm and difuses to the intercellular space through the different strata of the epidermis invading keratinocytes. Nuclei were stained with Sytox and the CY5 secondary antibody was employed for tyrosinase labeling. The image was obtained using a Leica TCS SP2 confocal microscope (Leica Microsystems, GmbH, Heidelberg, Germany). Image kindly provided by ME Jimenez-Capdeville and E Chi-Ahumada from the Department of Biochemistry, Faculty of Medicine, San Luis Potosí, SLP, Mexico.

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Rev Inves Clin. 2016;68:281-5 BRIEF REVIEW

Obstructive Sleep Apnea and Perinatal Risk

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ABSTRACT

Obstructive sleep apnea is characterized by total or partial interruptions of airflow during sleep, despite ongoing efforts to breathe. These pauses result from repeated upper airway obstructions that generate a systemic inflammatory condition with consequences for the endothelial function that increase the risk of cardiometabolic events. The prevalence of obstructive sleep apnea during pregnancy is greater than that observed in the general population and increases in the third trimester. Although limited, the information currently available supports the notion that obstructive sleep apnea is an independent, and potentially modifiable, risk factor for maternal and perinatal morbidity and mortality. Experimental and prospective studies in humans have demonstrated an association between obstructive sleep apnea and low birth weight. Endothelial dysfunction may be the link that underlies the association of obstructive sleep apnea with high perinatal risk. The information reviewed herein suggests that treating obstructive sleep apnea with positive-pressure devices could be an effective strategy for decreasing perinatal morbidity and mortality. (REV INVES CLIN. 2016;68:281-5)

Key words: Maternal risk. Pregnancy. Sleep apnea. Sleep-disordered breathing.

INTRODUCTION

Obstructive sleep apnea (OSA) is characterized by intermittent interruptions of breathing during sleep that may be total (apneas) or partial (hypopneas), and are caused by repeated collapses of the upper airway during sleep[1]. Apneas and hypopneas reduce oxygenation, which triggers the most widely studied damage mechanism in OSA patients, namely, hypoxemia-reoxygenation[2]. Other mechanisms that cause biological damage in OSA include an increase in sympathetic traffic caused by recurrent micro-arousals, variations in intrathoracic pressure, and episodes of hypercapnia-hypocapnia[2]. When OSA is accompanied by certain symptoms, especially excessive daytime somnolence, it is called obstructive sleep apnea syndrome (OSAS).

According to the 1993 Wisconsin Sleep Cohort Study[3], the population-based prevalence of OSAS is 2% in women and 4% in men. Similar figures have been reported for Latin America[4]. However, due to the epidemic of obesity in recent years, studies now demonstrate that the incidence of OSAS is greater, reaching levels as high as 5% in women and 14% in men[5].

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OBSTRUCTIVE SLEEP APNEA AND PREGNANCY

It has been amply demonstrated that OSAS causes cardiovascular diseases[6], motor vehicle- and work-related accidents[7,8], and poor quality of life[9]. Other associations proven recently include mortality due to cancer[10] and morbidity during pregnancy[11].

In 2014, an important research study analyzed a representative sample of maternal discharges from hospitals in the USA for the period 1998-2009[11]. The study including almost 56,000,000 events showed that patients with OSAS were at a higher risk for pregnancy related morbidities (Table 1), and that the risk was even greater when OSAS was associated with obesity. It is well known that obesity is associated with morbidity during pregnancy; in fact, the in-hospital mortality risk among pregnant women with OSAS was five times higher (95% CI: 2.4-11.5) than for women without this condition[11]. In addition, a systematic review and meta-analysis published by Pamidi, et al. confirmed that maternal OSAS was significantly associated with preeclampsia (OR: 2.34) and gestational diabetes (OR: 1.86)[12]. Another recent systematic review also supports the notion that moderate-to-severe sleep-disordered breathing is associated with most adverse perinatal outcomes, including low birth weight (OR: 1.75; 95% CI: 1.33-2.32), admissions to neonatal intensive care units (OR: 2.43; 95% CI: 1.61-3.68), intrauterine growth restriction (OR: 1.44; 95% CI: 1.22-1.71), and Apgar scores < 7 at one minute (OR: 1.78; 95% CI: 1.10-2.91)[13].

The prevalence of OSAS among women of reproductive age ranges from 0.7 to 7.0%, but increases to 11-20% in pregnant patients[11]. The highest prevalence of OSAS occurs in pregnant women who are also obese[14]. Other studies have demonstrated that the frequency of OSAS is three per 10,000 pregnancies (95% CI: 2.8-3.2), but this figure rose from 0.7 in 1998 to 7.3 in 2009, representing an annual increase of 24%[11]. The annual increase in obesity in the same period was 20%. This virtually parallel behavior of the incidence of OSAS during pregnancy and the prevalence of obesity suggests a possible causal association since it is well-known that obesity is the principal risk factor for developing OSAS[1]. Furthermore, a study by Pien, et al. found that by the third trimester of pregnancy, 26.7% of women had OSAS, and identified that both higher baseline body mass index and maternal age were risk factors for the onset of third-trimester OSAS among women without baseline sleep-disordered breathing[15].

Table 1. Associations between diagnosis of obstructive sleep apnea syndrome and maternal morbidity and mortality

Maternal morbidity/ mortality	Odds ratio	95% Confidence interval
Cardiomyopathy	9.01	7.47-10.87
Heart failure	8.94	7.45-10.73
Pulmonary edema	7.50	4.63-12.15
Eclampsia	5.42	3.29-8.92
In-hospital mortality	5.28	2.42-11.53
Pulmonary embolism	4.47	2.25-8.88
Hospital stay > 5 days	3.06	2.76-3.40
Cerebrovascular disease	2.93	1.07-8.04
Acute renal failure	2.73	1.69-4.41
Preeclampsia	2.50	2.19-2.85
Gestational diabetes	1.89	1.67-2.14
Gestational hypertension	1.28	1.08-1.52
Premature birth	1.20	1.06-1.37
Cesarean section	1.12	1.01-1.23

Unfortunately, there are no highly predictive strategies that are capable of properly identifying OSAS during pregnancy. The Berlin Questionnaire, a popular screening tool used in adults, was shown to have poor diagnostic performance in 43 pregnant women[16]. Other studies have similarly failed to demonstrate a causative association between the Berlin Questionnaire and OSAS[17], perhaps because the clinical manifestations of OSAS in pregnancy diverge from those seen under "typical" conditions. For instance, women report more fatigue and less snoring than men[18].

Table 2 shows data from Mexico's 2012 National Health and Nutrition Survey related to the frequency of overweight and obesity in Mexican women of reproductive age[19]. We believe that recognition of the coexistence of obesity with OSAS could be substantially contributing to the modest advances that have been made in reducing maternal mortality[20].

Regarding the potential effects of OSAS on newborns, we found that information is scarce. Studies using animal models have proven that intermittent hypoxemia

Table 2. Prevalence of overweight and obesity in Mexican women of reproductive age

Age (years)	Overweight BMI 25.0-29.9 kg/m^2		Obesity BMI ≥ 30.0 kg/m^2	
	Prevalence (%)	95% CI	Prevalence (%)	95% CI
12-19	23.7	22.1-25.5	12.1	10.9-13.4
20-29	30.6	28.5-32.8	24.0	22.0-26.1
30-39	38.1	36.2-40.1	37.3	35.3-39.4
40-49	37.6	35.3-40.0	46.1	43.8-48.4

Data from Mexico's 2012 National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición).
BMI: body mass index; 95% CI: 95% confidence interval.

causes low birth weight due, apparently, to a reduction of placental perfusion. Concerning humans, a recent cohort study of 234 patients showed that after adjusting for maternal parity, pre-pregnancy body mass index, ethnicity, gestational age, and newborn gender, the presence of OSA (documented objectively by polysomnography) was indeed found to be associated with low birth weight in relation to gestational age[21]. The main indicator of the severity of OSAS, the apnea-hypopnea index (> 10 events/hour), was also significantly associated with low weight for gestational age (OR: 2.65; 95% CI: 1.15-6.10). Also, the desaturation index (4%) was seen to be related to low weight for gestational age (OR: 1.17 for each event; 95% CI: 1.03-1.34). It is important to note that low birth weight has been shown to be clearly associated with a high risk for cardiac, metabolic, and neurological complications in adulthood[22]. An interesting recent study by Tapia, et al. demonstrated that ex-preterm schoolchildren have a tendency to suffer more OSAS later in life compared to the general population of similar age[23]. The findings reported previously by Rosen, et al. are similar[24].

MECHANISMS OF BIOLOGICAL DAMAGE LINKED TO OBSTRUCTIVE SLEEP APNEA SYNDROME

The physiopathological marker of OSAS is the collapse of the upper airway during sleep, caused by an imbalance between factors that promote it and others that oppose it[25]. The main factor that promotes this collapse is an increase in the extraluminal pressure secondary to an exaggerated deposition of fatty tissue in the parapharyngeal space. Negative intraluminal pressure is another factor that promotes collapse. In terms of the mechanisms that oppose such a collapse, the main factors are the downward traction force exerted by pulmonary volume combined with the contraction of the dilator muscles of the pharynx[1,25].

The possible mechanisms that may link OSAS to maternal morbidity and mortality are similar to those described in relation to cardiovascular damage[2]. It is well known that OSAS is associated with an increase in the sensitivity of chemoreceptors and a reduction in baroreflex sensitivity[2,26]. These mechanisms are at the very center of the biological damage caused by OSAS[2]. The exaggerated sensitivity of the chemoreceptors induces a state of generalized vasoconstriction that occurs not only while the person is asleep, but also persists during wakefulness[27]. This chemoreceptor dysfunction is mediated by oxygen-reactive substances that are generated in unusually high quantities during episodes of hypoxemia and reoxygenation[2]. The oxidative state of OSAS is associated with systemic inflammation and the activation of the endothelium[28], so it is possible that intermittent hypoxemia and increased sympathetic traffic generate an endothelial dysfunction that increases the risk of complications during pregnancy, such as preeclampsia and eclampsia. The effect of the oxidative state of OSAS on placental perfusion and the endothelial function could be the factor that underlies the association with hypertensive states during pregnancy[11]. Poor regulation of blood pressure secondary to reduced baroreflex sensitivity may well be another factor related to the vascular complications observed in pregnant women with OSAS[27]. However, additional mechanisms, such as insulin resistance, dyslipidemia, reduced availability of

nitric oxide, and over-activation of the sympathetic nervous system, among others, could also cause vascular damage in patients with OSAS[2,28-32].

TREATMENT

Continuous positive airway pressure (CPAP) is the preferred treatment option for patients with moderate-to-severe OSAS[33]. Positive pressure prevents collapse of the upper airway by forming a kind of "pneumatic splint" that normalizes the respiratory pattern. Until now, the benefits of CPAP for patients with *OSAS* have been shown to be clear and consistent[6,34,35], since numerous studies have demonstrated that CPAP reduces the incidence of both fatal and non-fatal cardiovascular events compared to observations in the general population[6]. The effectiveness of nasal CPAP during pregnancy, however, has been little studied and most information comes from small case series. A study of 12 pregnant women diagnosed with sleep-disordered breathing carried out by Guilleminault, et al. demonstrated that nasal CPAP is a safe and effective treatment during pregnancy[36]. Furthermore, in a small randomized study conducted by Poyares, et al. evaluating the effectiveness of nasal CPAP in pregnant women with hypertension and chronic snoring, the authors found that eight weeks of CPAP use was associated with better blood pressure control when compared to standard prenatal care[37].

PERSPECTIVES AND CONCLUSIONS

The growing problem of obesity in women of reproductive age in Mexico leads us to predict that the frequency of OSAS during pregnancy will increase significantly in coming years. Today, a strong body of evidence supports OSAS as an independent risk factor of maternal and perinatal morbidity and mortality. Although the nasal CPAP strategy has potentially favorable implications for reducing negative outcomes during pregnancy, implementing it as a public health approach is not feasible at this time, simply because the sleep medicine infrastructure in Mexico's health system is insufficient. As a result, any public health policies designed to decrease OSAS-related morbidity or mortality during pregnancy through the use of sleep laboratories attended by qualified physicians are inappropriate. Thus, it is important to implement simple and effective means. For example, implementing better weight-control programs before and during pregnancy may be a particularly useful and cost-effective strategy for reducing maternal morbidity associated with OSAS.

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Rev Inves Clin. 2016;68:286-91 BRIEF COMMUNICATION

The Type of *Trypanosoma Cruzi* Strain (Native or Non-Native) Used as Substrate for Immunoassays Influences the Ability of Screening Asymptomatic Blood Donors

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ABSTRACT

Background: The origin (native or non-native) of *Trypanosoma cruzi* strains used as substrate for immunoassays may influence their performance. **Objective:** To assess the performance of an immunoassay based on a native *T. cruzi* strain compared to another based on non-native *T. cruzi* strains, in asymptomatic blood donors from Mexico. **Methods:** Serum samples from a tertiary referral center were tested by both ELISA-INC9 (native) and Chagatest (non-native) assays. All reactive serum samples were further analyzed by indirect immunofluorescence. **Results:** Sera from 1,098 asymptomatic blood donors were tested. A 4.3 and 0.7% serum reactivity prevalence was observed using ELISA-INC9 and Chagatest, respectively (kappa = 0.13; −0.11 to 0.38). Subsequently, indirect immunofluorescence analyses showed higher positivity in serum samples reactive by ELISA-INC9 compared to those reactive by Chagatest (79 vs. 62.5%; $p < 0.001$). Furthermore, out of the 47 positive samples by both ELISA-INC9 and indirect immunofluorescence, only four (8.5%) were reactive in Chagatest assay. Meanwhile, four (80%) out of the five positive samples by both Chagatest and indirect immunofluorescence were reactive using ELISA-INC9. **Conclusion:** Immunoassays based on a native *T. cruzi* strain perform better than those based on non-native strains, highlighting the need to develop and validate screening assays in accordance to endemic *T. cruzi* strains. (REV INVES CLIN. 2016;68:286-91)

Key words: Chagas disease. *Trypanosoma cruzi*. Immunoassay.

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INTRODUCTION

Chagas disease is a chronic infection by the protozoan flagellate *Trypanosoma cruzi*[1]. Vector-borne transmission to humans is the main route of infection followed by transfusion-associated (i.e. iatrogenic) transmission[2]. Despite an estimated 1.1-2.0 million inhabitants being infected by Chagas disease, Mexico finally included mandatory serological screening of blood derivatives for *T. cruzi* as late as 2012[3]. This is relevant since transfusion medicine continues to have a key role in medical care. For instance, in Mexico a total of 1,765,681 allogeneic units of blood were collected in 2011 from asymptomatic blood donors, and considering a nationwide seroprevalence ranging from 0.17 to 3.1% of blood donations, up to 54,736 of these hemoderivatives could have been potentially infectious[4-6]. In addition, millions of at-risk individuals have migrated to cities in endemic regions as well as to countries outside the endemic range, thus urbanizing and globalizing the problem of Chagas disease and giving rise to new epidemiological and public health problems to places where trypanosomiasis is not endemic[7,8].

Currently, prevention for transfusion-associated transmission of Chagas disease is based on detecting antibodies specific to several parasite antigens using serological tests. Enzyme-linked immunosorbent assays (ELISA), indirect hemagglutination chemiluminescence (IHA), and immunofluorescence assays are the most commonly employed[9]. These tests use antigens either from parasite lysates or recombinant proteins, which, if coupled with adequate antigen processing, can achieve acceptable sensitivity. However, a single test is not sufficiently sensitive or specific for diagnosis, and therefore diagnostic accuracy is further improved when a second test (based on a different principle from the first used) is performed as currently recommended by the World Health Organization (WHO)[10]. Further, the correct identification of the endemic *T. cruzi* strains to the region of study is of importance due to strain heterogeneity, which may lead to biologic and genetic differences that may potentially influence serological identification of *T. cruzi* strain-specific antibodies[11]. This emphasizes on the importance of selecting the ideal strain for parasitic antigen preparation, particularly considering its potential effects on the performance of serologic diagnostic tests, especially when employed in a region with endemic strains different to that used for the standardization process of the test.

In 2001, the INC9 strain was isolated in a sample from a patient from the state of Guerrero presenting with chronic chagasic cardiomyopathy, who was treated at the Instituto Nacional de Cardiología in Mexico City, Mexico. This strain was further characterized as MHOM/MX/2001/INC9 according to the standard nomenclature. Analyses for *T. cruzi* mini-exon gene sequence (5 ' GTGTCCGCCACC TCCTTCGGGCC3 ') using the method proposed by Souto, et al.[12], and confirmed by fluorescent DNA sequencing, allowed its identification as a discrete typing unit (DTU) I[13]. This result agreed with previous reports showing *T. cruzi* DTU I as the prevalent type in Mexico, regardless of the geographic region of acquisition[14], in contrast to the prevalent DTU II strains observed in South America[15]. The parasitic lysate from INC9 also has proved to be an adequate source of antigenic peptide substrates for the serologic identification of anti-*T. cruzi* antibodies, both in asymptomatic blood donors and in patients with chronic chagasic cardiomyopathy[16].

This study was aimed to evaluate the performance of an ELISA test based on whole protein extracts from epimastigotes of INC9 strain of *T. cruzi* compared to an ELISA test based on recombinant antigens from *T. cruzi* strains endemic to South America, in asymptomatic blood donors from Mexico.

MATERIALS AND METHODS

Subjects

This study included serum samples of asymptomatic blood donors from the Instituto Nacional de Cardiología in Mexico City. Although the local authorities collated clinical and demographic characteristics from blood donors, these data were not available for publication. The study protocol was approved by the institutional review board and ethics committee of the institute and was performed following the principles of the Declaration of Helsinki and local regulations. In addition, an informed consent was obtained from all the individuals.

Trypanosoma cruzi cultures and antigenic whole protein extract

Isolated epimastigotes of the INC9 strain of *T. cruzi* (native strain, TcI; see below) were cultured in an

axenic culture using liver infusion tryptose medium supplemented with 10% fetal bovine serum with prior inactivation at 56°C for 30 minutes and 25 mg/ml of hemin[16]. Cultured parasites were processed at the exponential growth phase. For the antigenic whole protein extract elaboration, parasites were collected by centrifugation at 1000 x g for 30 minutes at 4°C, then washed and centrifuged two times using a phosphate-saline buffer (PBS), pH 7.2. The pellet was suspended in PBS, pH 7.2 (1-3 times packed volume of pellet) and a protease inhibitor cocktail was added. The parasite suspension underwent 8-10 rounds of sonication for periods of one minute. Finally, this mix was centrifuged at 10,000 x g for 30 minutes at 4°C and the supernatant (total extract) was collected. After determining the protein concentration, the extract was stored frozen at −20°C in aliquots of 0.5 ml each.

Immunoassay based on a native *Trypanosoma cruzi* (ELISA-INC9) strain

A modified version of a previously validated ELISA method was used for serological screening of anti-*T. cruzi* antibodies[17]. Briefly, ELISA polystyrene microplate (Costar, Cambridge, MA, USA) wells were incubated with 1 µg of antigenic whole protein extract from *T. cruzi* (INC9 strain) and 200 µl of carbonate-bicarbonate buffer, pH 9.6, 0.05 M (J.T. Baker, Phillipsburg, NJ, USA). After one hour incubation at 37°C, microplates were washed five times with 215 µl of PBS (J.T. Baker)-Tween 20 (Sigma-Aldrich, St Louis, MO, USA) 0.05%. Wells were incubated with blocking solution (PBS-Bovine serum albumin 0.5%) for 20 minutes at 37°C. Serum samples were diluted 1:200 in PBS and anti-human IgG conjugated to peroxidase (Invitrogen, Carlsbad, CA, USA) was diluted 1:10,000 in PBS. The developing process used O-phenylenediamine (OPD; Sigma-Aldrich) as substrate for peroxidase and a 10-minute incubation time at room temperature. A 5N sulfuric acid diluted solution (Sigma-Aldrich) was employed for stopping the reaction, followed by absorbance determination at 450 nm[17].

The cut-off value for positivity was set after analyzing serum samples from five healthy blood donors known to be non-reactive for anti-*T. cruzi* antibodies by three different assay methods, Western blot, commercially available ELISA, and IIF assays (see below). The cut-off was set to the mean value plus three standard deviations, and it was calibrated in each ELISA plate.

Immunoassay based on non-native *Trypanosoma cruzi* strains

The Chagatest ELISA test v.3.0 (Wiener Lab, Rosario, Argentina) was used for the qualitative serological screening of anti-*T. cruzi* antibodies, following the instructions provided by the manufacturer. In this commercially available assay, antigens are obtained by DNA recombinant techniques starting from specific proteins from the epimastigote and trypomastigote stages of *T. cruzi* strains endemic in South America.

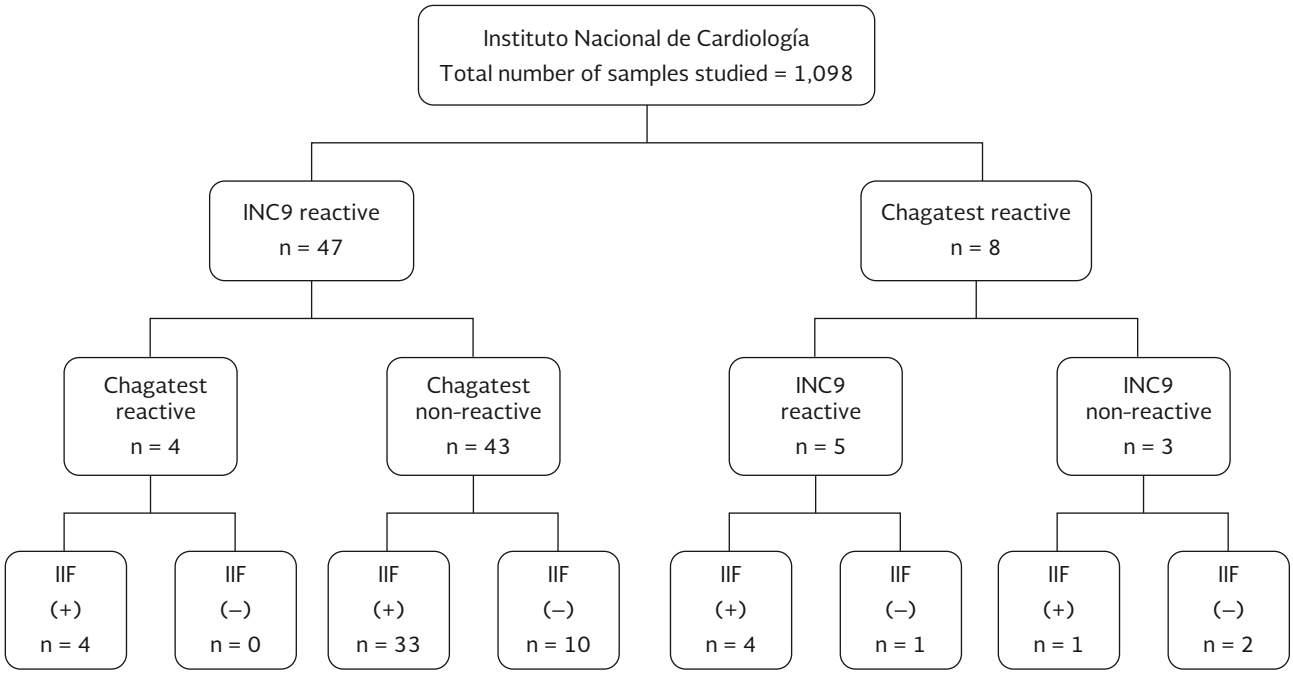
Indirect immunofluorescence assays

Indirect immunofluorescence (IIF) assays were used to confirm the positivity of reactive sera by any of the ELISA assays. For analysis of fixed cells, *T. cruzi* epimastigotes were allowed to settle onto a microscopy slide stored at −20°C until used. Prior to test, microscopy slides were thawed and hydrated with PBS. Patients' serum samples and known positive controls were diluted 1:40 in PBS. Positive control samples were obtained from known patients with chronic chagasic cardiomyopathy reactive to anti-*T. cruzi* antibodies by three different assay methods (Western blot, commercial ELISA, and IIF assays). The anti-human IgG antibody conjugated to FITC was diluted 1:150 in PBS. Microscopy slides were washed three times with PBS after an overnight incubation at 4°C, fixed with 100 µl 0.01 M (1X) PBS solution, and dried for 18 hours. Finally, indirect immunofluorescence analysis was done using epifluorescence microscopy.

Statistical analysis

Proportions and percentages were used to describe categorical data and the differences were assessed using the chi-square or the Fisher's exact tests, as appropriate. Meanwhile, the degree of consistency between the different tests was determined by the un-weighted Cohen's kappa coefficient with 95% confidence intervals (95% CI). A p value < 0.05 was set for significance. The GraphPad Prism v 4.02 (GraphPad Software, La Jolla, CA, USA) software and the online calculator (http://www.redcaspe.org) from the CASPe (Critical Appraisal Skills Program Español) network were used for calculations.

Figure 1. Results of serum samples from asymptomatic blood donors. Serum reactivity by ELISA-INC9, Chagatest ELISA, and indirect immunofluorescence assays. IIF: indirect immunofluorescence.



RESULTS

A total of 1,098 serum samples of asymptomatic blood donors from the blood bank of the Instituto Nacional de Cardiología were tested using both the Chagatest and the ELISA-INC9 assays (Fig. 1). Reactivity was observed in 47 samples by ELISA-INC9; meanwhile, Chagatest displayed reactivity in eight samples (4.3% vs. 0.7%; $p < 0.0001$). Further, only four reactive samples were common to both assays, resulting in a slight agreement as disclosed by a kappa index of 0.13 (95% CI: −0.11 to 0.38).

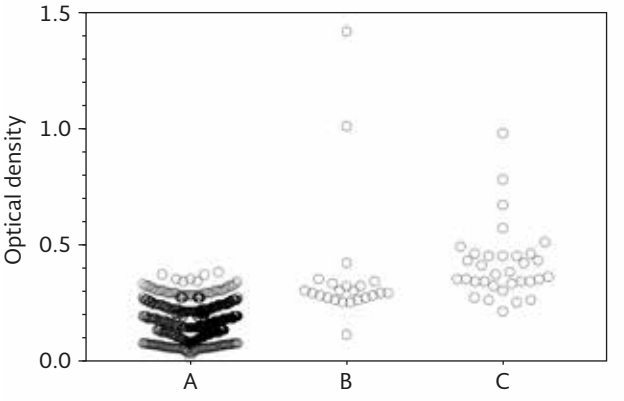
Serum reactivity was subsequently confirmed using IIF. Thirty seven out of 47 reactive samples by ELISA-INC9 were confirmed to be positive by IIF; in contrast, only 5 out of eight reactive samples by Chagatest were confirmed to be positive by IIF (79% vs. 62%; $p = 0.1$). Overall, only 4 (10.8%) out of the 37 positive samples by both ELISA-INC9 and IIF were reactive in the Chagatest assay, and four (80%) out of the five positive samples by both Chagatest and IIF were reactive in the ELISA-INC9 (Fig. 1).

Figure 2 shows the optical density (OD) values for each serum sample tested in the ELISA-INC9. Samples were grouped as follows: (A) ELISA-INC9 and IIF negative; (B) ELISA-INC9 positive but IIF negative; and (C) both tests shown to be positive. Notably, the mean OD was similar in groups B and C, and significantly higher when compared with the OD observed in group A.

DISCUSSION

Transfusion medicine has contributed to a decrease in mortality and improved quality of life of many individuals suffering from a variety of diseases, and thus it is considered as a great achievement of modern therapeutics[18]. In spite of this, Chagas disease, along with other disorders, has been recognized as having transfusion-associated transmission, hampering the therapeutic purpose of transfusion medicine[19]. Furthermore, Chagas disease and its severity seem to vary according to different geographic regions and hence may be considered as a major problem for transfusion medicine in endemic regions. In December 2002, WHO guidelines incorporated high sensitivity tests for the screening of anti-*T. cruzi* antibodies in blood and blood components to be used for transfusion as an important approach to address

Figure 2. Total values in optical densities for sera from blood donors tested in the ELISA-INC9. Serum samples are grouped as follows: **A:** ELISA-INC9 and IIF negative; **B:** ELISA-INC9 positive but IIF negative; **C:** both tests shown to be positive. The mean cut-off point was determined to be 0.30 ± 0.05 optical density. IIF: indirect immunofluorescence.



this problem[2]. However, currently there is no single widely accepted serologic reference standard for anti-*T. cruzi* antibody screening.

In the present study, we sought to determine whether the results of an immunoassay based on antigenic substrate from an autochthonous or native strain would resemble those of a commercially available immunoassay (based on non-native strains) and at the same time comply with WHO recommendations for anti-*T. cruzi* antibody screening in asymptomatic blood donors from Mexico. Overall, we found different seroprevalence when using either native or non-native based assays, and observed only a slight agreement between these two tests. In this vein, we found a higher seroprevalence using ELISA-INC9 in asymptomatic blood donors (overall seroprevalence of 8.8%) as compared to 0.7% for the Chagatest. It is important to note that 79% of serum samples reactive by ELISA-INC9 were subsequently confirmed to be positive by IIF. On the other hand, IIF assessment of reactive samples using the Chagatest assay resulted in a 62.5% confirmation. Though these figures were not statistically different, it is important to note that there were positive sera by both ELISA-INC9 and IIF that were non-reactive using the Chagatest assay. In fact, only four out of the 37 positive sera by ELISA-INC9 and IIF were reactive by the Chagatest ELISA as well, resulting in an alarming 89% non-detection rate of positive individuals by the Chagatest assay. In contrast, four out of the five positive sera by the Chagatest ELISA and IIF were also reactive using ELISA-INC9 (89% vs. 20% non-detection rate of positive sera; p= 0.002). A smaller study from Mexico has previously emphasized on this problem, where the use of antigens from Mexican *T. cruzi* strains in an ELISA assay were able to correctly identify as positive known sera from patients with trypanosomiasis in 100% of cases, whereas the use of antigens from Argentinian strains correctly identified only 18% of individuals[20].

In this regard, the confidence of serologic tests depends on diverse factors that include cross-reactivity with other related protozoa, low anti-*T. cruzi* antibody count, or serum samples inadequately processed[21-23]. In addition, a dimorphism in the 24S alpha rRNA target region has allowed the characterization of DTU I and II, which probably resulted from long-term clonal evolution of *T. cruzi*[24,25]. Interestingly, multi-locus markers have further showed a total of six DTUs, one corresponding to *T. Cruzi* I and the others to subdivisions within *T. cruzi* II, IIa-e[26-28]. However, by consensus, currently recognized nomenclature for *T. cruzi* now classifies strains into six DTUs, *T. cruzi* I-VI[29]. In this vein, TcI and TcII are now used to name the previously known DTU I and DTU IIb, respectively. For our study, it is important to recognize that Mexican TcI strains are a homogeneous group closely related to each other and considered as the primary agents of serum reactivity in Mexico[30]. On the other hand, Mexican TcII are not closely related to TcII strains circulating in domestic cycles in Argentina, Brazil, Bolivia, and Chile[14]. Thus, the use of antigenic substrate from a native strain (TcI) may have given an advantage to ELISA-INC9 over the commercial ELISA assay based on autochthonous South American strains (where non-TcI strains predominate) by potentially providing different antigenic determinants key for screening these two particular samples and for yielding a higher seroprevalence[16,20].

Concurrently, our ELISA-INC9 findings indicate that by using antigenic substrate from a native *T. cruzi* strain, it is possible to achieve identification of a higher number of positive individuals and, thus, less infectious blood and blood derivatives available for transfusion therapy, contributing to decrease transfusion-associated transmission of Chagas disease. On the contrary, the screening based on non-TcI strains from South

America, which exhibit little relationship with *T. cruzi* strains from Mexico, could be inadequate for seroprevalence surveys in the Mexican population.

The present study has several limitations. First, parasite isolation by xenodiagnosis in those cases with serum reactivity in the ELISA-INC9 method would have further confirmed the performance of the test by determining true positives. Conversely, an alternative approach would include the use of well-characterized serum samples from subjects known to be infected with different *T. cruzi* strains. Second, in an attempt to support the previously validated immunologic screening tests employed, both the home-made ELISA and IIF assays were standardized employing the same *T. cruzi* strain (INC9). Finally, the possibility of cross-reaction with antibodies elicited by other pathogens (such as *Leishmania*) was not assessed.

In conclusion, our results suggest that each geographic region should develop and validate its own screening immunoassays for anti-*T. cruzi* antibodies in accordance to endemic *T. cruzi* strains.

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PERMANYER

Rev Inves Clin. 2016;68:292-8 ORIGINAL ARTICLE

The Neuroprotective Effect of Erythropoietin in Rat Hippocampus in an Endotoxic Shock Model

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ABSTRACT

Background: Sepsis is characterized by an early systemic inflammation in response to infection. In the brain, inflammation is associated with expression of pro-inflammatory cytokines (e.g. tumor necrosis factor-α, interleukin-1β and interleukin-6, among others) that may induce an overproduction of reactive oxygen and nitrogen species. The constitutive expression of cytokines in the brain is low, but may be induced by various stimuli, including lipopolysaccharide, which causes neuronal damage. Erythropoietin, among other effects, acts as a multifunctional neurotrophic factor implicated in neurogenesis, angiogenesis, vascular permeability, and immune regulation in the central nervous system. In an experimental model of endotoxic shock, we studied the neuroprotective capacity of erythropoietin in the rat hippocampus and compared with melatonin, a neurohormone with an important antioxidant and immunomodulatory effect. **Methods**: In 21-day-old male Wistar rats divided into eight groups, we administered by intraperitoneal injection lipopolysaccharide, erythropoietin, melatonin, or combinations thereof. The hippocampus was dissected and morphological (histological analysis) and biochemical (cytokine levels) studies were conducted. **Results:** The number of dead neuronal cells in histological sections in groups treated with lipopolysaccharide was higher compared to the erythropoietin group. There was a greater decrease (70%) in interleukin-1β concentrations in rats with endotoxic shock that received erythropoietin compared to the lipopolysaccharide group. **Conclusions:** The neuronal cell loss caused by endotoxic shock and interleukin-1β levels were reduced by the administration of the hematopoietic cytokine erythropoietin in this experimental model. (REV INVES CLIN. 2016;68:292-8)

Key words: Erythropoietin. Endotoxic shock. Neuroimmunology. Neuroinflammation. Oxidative stress.

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INTRODUCTION

Endotoxic shock and sepsis represent an important clinical challenge worldwide[1]. Sepsis is characterized by early systemic inflammation in response to an infection and is associated with hypoperfusion, followed by tissue injury and subsequent organ damage[1-3]. The central nervous system (CNS) plays an important role in the production of cytokines and other immune factors[3]. In the brain, inflammation is associated with glial cell activation and proliferation, usually following an acute inflammatory response. Indeed, while the constitutive expression of cytokines in the brain is low, it can be induced by various stimuli, including lipopolysaccharides (LPS)[4,5].

In the CNS, LPS induces the migration of activated lymphocytes and other immune cells, which cross the blood-brain barrier (BBB) or blood-cerebrospinal fluid barrier[6]. The transmigration of primed cells induces the BBB endothelium to relax its tight junctions, allowing the passage of cells carried in the blood into the CNS[6]. This process activates neurons and glial cells to express pro-inflammatory cytokines (e.g. tumor necrosis factor alpha [TNF-α] and interleukins IL-1β and IL-6, among others), stimulates clusters of differentiation (CDs) like CD200 (neurons) and CD200R (microglia), as well as promoting the expression of adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1)[6]. Elevated concentrations of TNF-α, IL-1β, and IL-6 may induce an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), both of which can cause injury during development in susceptible areas of the CNS, including the cerebral cortex and hippocampus[7].

Studies in rats treated with LPS show alterations in the BBB and morphological changes in the hippocampus associated with neuronal death[8,9]. The hippocampus, specifically the CA1 region, is more susceptible to damage than other areas in the brain. Some features of this region that make it more vulnerable compared to other areas of the hippocampus or other brain regions include: (i) the highest density of AMPA and NMDA receptors that makes it more susceptible to excitotoxic damage caused by glutamate released by glutamatergic projections from CA3[10]; (ii) the lower density of blood vessels and increased susceptibility of these to show BBB alterations caused by ischemia[11]; and (iii) the increased vulnerability of astrocytes in the CA1 region to the damage caused by free radicals in the mitochondria[12].

Thus, it is important to define new therapies for patients with endotoxic shock that can modulate the inflammatory immune response of the brain at early stages of maturation and development, which are critical for memory and learning. Interestingly, it appears that the alterations elicited by endotoxic shock may be prevented by the hematopoietic cytokine erythropoietin (EPO), which is produced in the liver, kidney, heart, and brain[13-17], and by melatonin (MLT), synthesized not only by the pineal gland but also in retina, gastrointestinal tract, thymus, and bone marrow, among others. The protective effects of MLT against sepsis are suggested to be due to its antioxidant immunomodulating and inhibitory actions against the production and activation of pro-inflammatory mediators[18-20]. Novel biological activities of EPO have recently been described, such as those of a multifunctional neurotrophic factor implicated in neurogenesis, angiogenesis, vascular permeability, and immune regulation in the CNS[13,17,21]. The EPO does not normally cross the BBB but, during an inflammatory process such as that induced by LPS, the barrier's permeability is altered and, as cytokine levels increase, these are able to cross the BBB[17,22]. The aim of the present study was to evaluate the neuroprotective capacity of EPO in the rat CNS (hippocampus) following endotoxic shock.

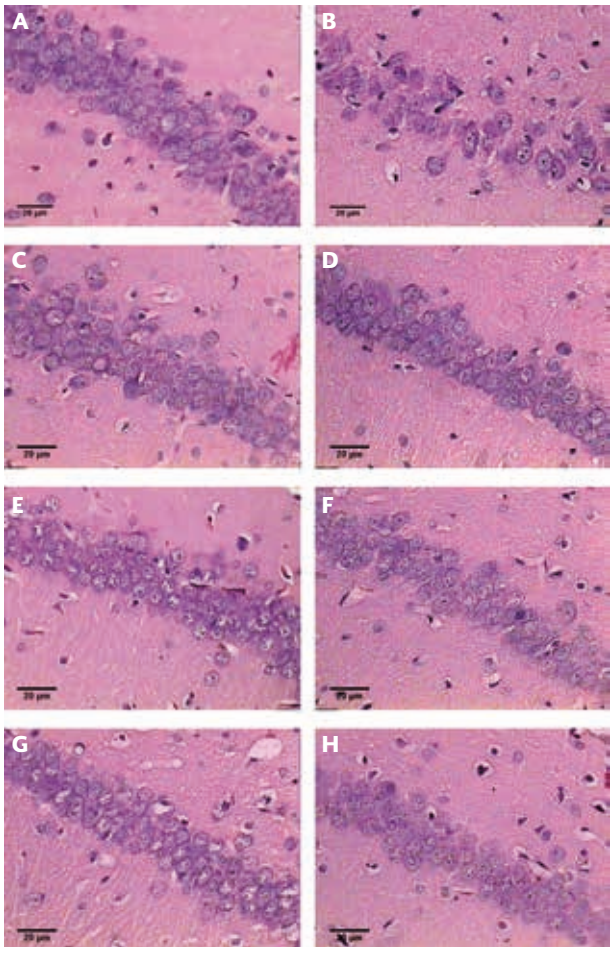
MATERIALS AND METHODS

Experimental groups

All experiments were performed in 21-day-old male Wistar rats maintained on a 12/12 hour light/dark cycle at an ambient temperature of 22 ± 2°C, with food and water *ad libitum*. Experiments were carried out following the Mexican guidelines for handling laboratory animals (Norma Oficial Mexicana para el Manejo de Animales de Laboratorio, NOM-062-ZOO-1999).

Animals were divided into eight groups (n = 10 each): (i) sham (intact rats); (ii) LPS (lipopolysaccharide from *Escherichia coli* O111:B4 15 mg/kg^{-1}; Sigma St. Louis, MO, USA); (iii) MLT (melatonin 10 mg/kg^{-1}; Sigma St. Louis, MO, USA); (iv) EPO (erythropoietin

Figure 1. Representative photomicrographs of the hippocampal CA1 area in 21-day-old rats: **A:** sham; **B:** lipopolysaccharide (LPS); **C:** melatonin (MLT); **D:** erythropoietin (EPO); **E:** LPS + EPO; **F:** LPS + MLT; **G:** MLT + EPO; **H:** LPS + MLT + EPO. Paraffin-embedded tissue sections (8 µm) stained with hematoxylin and eosin and examined under an optic microscope (40x magnification).



5,000 U/kg^{-1}; PISA, Mexico); (v) LPS + EPO (LPS 15 mg/kg^{-1} + EPO 5,000 U/kg^{-1}); (vi) LPS + MLT (LPS 15 mg/kg^{-1} + MLT 10 mg/kg^{-1}); (vii) MLT + EPO (MLT 10 mg/kg^{-1} + EPO 5,000 U/kg^{-1}); and (viii) LPS + MLT + EPO (LPS 15 mg/kg^{-1} + MLT 10 mg/kg^{-1} + EPO 5,000 U/kg^{-1}). All chemicals were administered by intraperitoneal injection.

We used a concentration of LPS (LD75) to generate clinical signs and symptoms of acute shock and assess the activity of EPO. The MLT was used as a control due to its immunomodulatory activity and to compare the results with those of EPO in the endotoxic shock model.

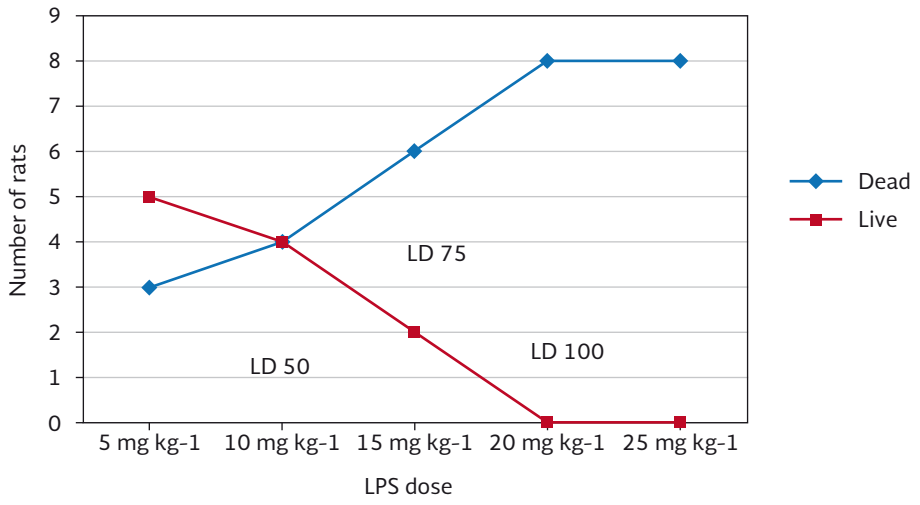
Histological methods

To characterize the effect of LPS and the other treatments in the hippocampal neurons, half of the rats (n = 5) in each group (Sham, LPS, MLT, EPO, LPS + EPO, LPS + MLT, MLT + EPO, and LPS + MLT + EPO) were studied histologically. Rats were anesthetized with a lethal dose of sodium xylazine (5 mg kg^{-1}) and ketamine (80 mg kg^{-1}) administered by intramuscular injection, and were then perfused with 180 ml of 0.9% NaCl containing 10 Ul^{-1} of heparin and 0.01% procaine at body temperature for about five minutes. Animals were then perfused for 10 minutes with 280 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), at a perfusion pressure of 140 cm H_2O[23]. The perfused brains were embedded in paraffin and serial coronal sections (10 µM) including the dorsal hippocampus (CA1 area) were stained with hematoxylin-eosin to visualize the degree of cell damage (Fig. 1). Cells were counted in 4-5 fields per section from four sections taken from each of the five animals studied from each group. Cells were counted under a light microscope (Leica DME, 40x magnification) equipped with an analog photomicroscope system, using ImageJ software[24]. Neurons with a characteristic regular morphology, regular cell membrane outline, homogeneous cytoplasm, and well-defined nucleus were classified as normal cells (live neurons). Cells that had any fragmentation, shrinkage, basophilic cytoplasm, pyknotic nucleus, swelling, ghost form, or vacuolization were classified as damaged or morphologically abnormal cells (dead neurons)[25]. Results were expressed as the number of dead neurons (cell death).

Analysis of inflammation markers

The remaining five animals within each group were used for cytokine determination. Rats were sacrificed by decapitation 12 hours after inoculation; it has been shown that between eight and 12 hours after administration of LPS, endotoxic shock ensues and proinflammatory cytokine levels increase. The hippocampus was dissected out and maintained at −20°C. Tissue was homogenized in PBS containing a protease inhibitor cocktail (Calbiochem-Novabiochem, San Diego, CA) at 500 µl per 50 mg of tissue. Protein expression was evaluated using enzyme-linked immunosorbent assays (ELISA) to measure rat TNF-α, rat IL-1β/IL-1F2, and rat IL-6 (all ELISA kits were obtained from Quantikine ELISA kit, R&D Systems, Minneapolis, MN, USA).

Figure 2. Dose-response curve to calculate the lethal dose $(LD)_{50}$, LD_{75} and LD_{100} of lipopolysaccharide (LPS).



Absorbance was measured at 450 nm and wavelength correction was performed to 540 or 570 nm, using a Bio-Rad Microplate Reader Model 550.

Statistical analysis

Data are presented as means and standard error. Due to the non-parametric distribution of the data, as shown by the Kolmogorov-Smirnov test ($p < 0.05$), the differences between groups were assessed using Kruskal-Wallis and Mann-Whitney *U* tests with significance set at < 0.05. All statistical analyses were conducted with the Statistical Program for Social Sciences v22.0 (SPSS, Inc.; Chicago, ILL, USA).

RESULTS

Lipopolysaccharide dose-response curve

The appropriate dose of LPS to be administered intraperitoneally was calculated in a dose-response curve, generated by administering different LPS concentrations (5, 10, 15, 20, or 25 mg/kg^{-1}) to groups of eight rats each (Fig. 2). From these curves, the LD50 (10 mg/kg^{-1}), LD75 (15 mg/kg^{-1}), and LD100 (20 mg/kg^{-1}) were calculated to induce endotoxic shock. Clinical symptoms and manifestations of endotoxic shock in rats included rough hair, profuse diarrhea, conjunctivitis, and photophobia. During the necropsy, we observed internal bleeding and petechiae in liver and lung. The final concentration of LPS used in the model was the LD75.

Neuronal death

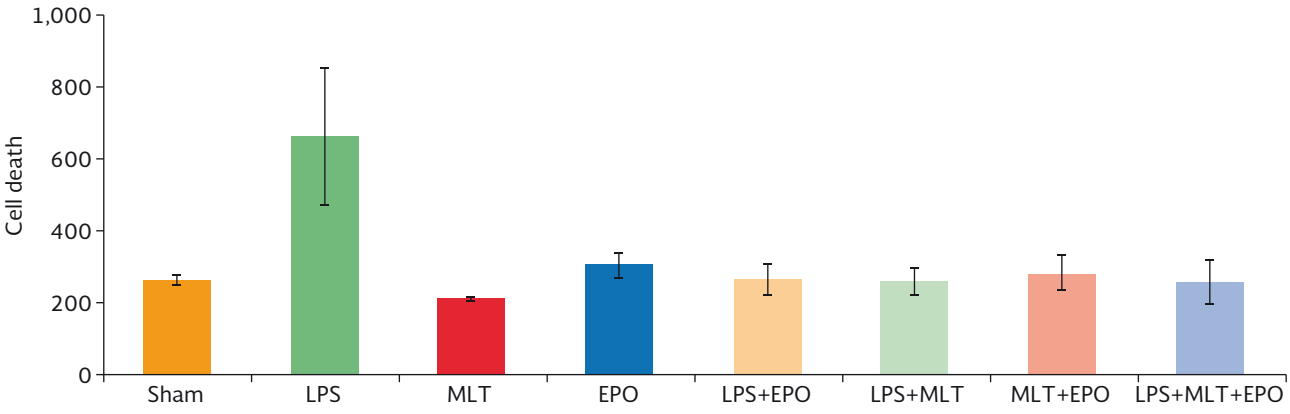
When we analyzed cell death in the hippocampal CA1 area (Fig. 3), there was a mean ($\bar{x}$) of 265.25 dead neurons in sections from the control group, which represented 59.97% of the cell loss per mm^2 observed in the experimental group that developed endotoxic shock (LPS group, $p \leq 0.05$). In the MLT group ($\bar{x}$ = 215.50) there were significantly fewer live neurons compared to the LPS group (67.47% reduction, $p \leq 0.05$), while the EPO group ($\bar{x}$ = 305.75) and LPS + EPO group ($\bar{x}$ = 266.00) displayed 53.85 and 59.85% less neuronal death, respectively ($p \leq 0.05$ compared to the LPS group).

In the experimental groups that received MLT alone or in combination (LPS + MLT, = 261.25; MLT + EPO, = 284.50; and MLT + LPS + EPO, = 259.00), the proportion of neuronal death was lower than in rats that received LPS alone, representing a reduction of 60.57, 57.06, and 60.91%, respectively ($p \leq 0.05$).

Interleukin-1β levels

The minimum detectable dose (MDD) of rat IL-1β is typically < 5 pg/ml^{-1}. The mean concentration of IL-1β in the rats that developed endotoxic shock was 1,166 ± 339.4 pg/ml^{-1}, whereas this cytokine was

Figure 3. Cell death in the CA1 region of the hippocampus of 21-day-old rats. The cells were counted in paraffin sections (8 µm) stained with hematoxylin and eosin. Data are shown as a mean number of cells per mm^2 ± standard error of the mean of five animals. Statistically significant differences were observed between the lipopolysaccharide (LPS) group and all other groups ($p \leq 0.05$). There were statistically significant differences between the group with melatonin (MLT) and LPS groups (LPS + erythropoietin [EPO], LPS + MLT, and LPS + MLT + EPO; $p \leq 0.05$).



undetectable in the sham and MLT groups, representing up to 100% lower levels of this cytokine compared to the LPS group ($p \geq 0.0001$). In the rats that received erythropoietin (EPO group), we obtained a mean value of 94.3 ± 31.2 pg/ml^{-1} of IL-1β, which was 91.91% lower than in the LPS group ($p \geq 0.0001$). In the rats that received LPS + EPO, the mean levels were = 325.8 ± 35.5 pg/ml^{-1}, 72.07% lower than in those that received LPS alone (1,166.5 ± 339.4 pg/ml^{-1}, $p \geq 0.005$), whereas in the animals that were subjected to endotoxic shock and received MLT (LPS + MLT group), the mean IL-1β levels were 245 ± 56.0 pg/ml^{-1}, 78.95% lower than the levels of rats that received LPS alone ($p \geq 0.002$). Consistent with the earlier results, the levels of IL-1β in the rats that received MLT + EPO were = 4.2 ± 4.2 pg/ml^{-1}, 99.63% lower than in the animals that received the endotoxic shock alone ($p \geq 0.0001$). Finally, in the rats that received MLT + LPS + EPO, the mean IL-1β levels were 24.9 ± 15.5 pg/ml^{-1}, 97.86% less than in the rats that received LPS alone (1,166 ± 339.4 pg/ml^{-1}, $p \geq 0.0001$; Fig. 4).

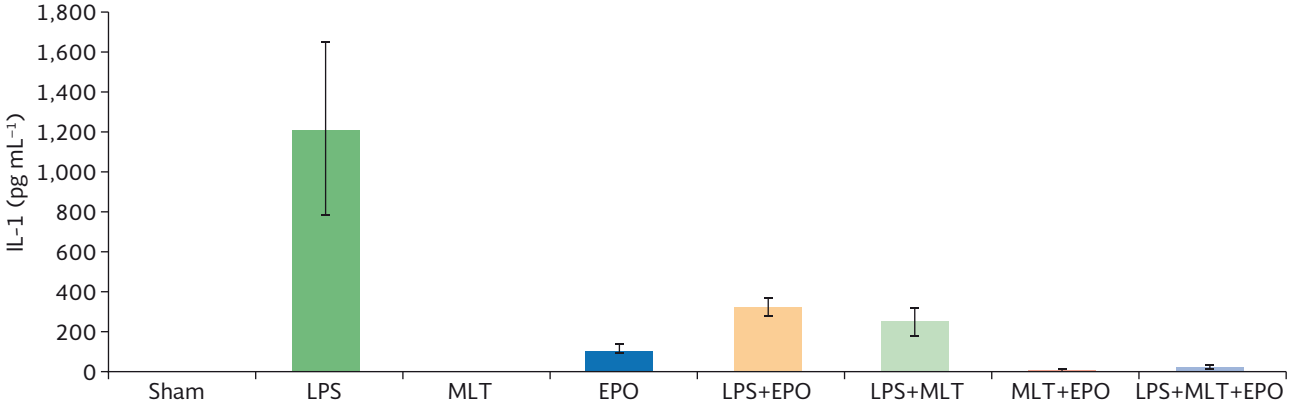
Tumor necrosis factor-α and interleukin-6 levels

In contrast to IL-1β, TNF-α and IL-6 were below the detection limit in this model of endotoxic shock. The minimum detectable dose (MDD) of rat IL-6 ranges from 14 to 36 pg/ml^{-1}, and the MDD of rat TNF-α is typically < 5 pg/ml^{-1}.

DISCUSSION

Oxygen deficiency in tissues results in the generation of EPO, an increase in the expression of the EPO receptor, EPO-R, in kidney and liver. During a severe hypoxia event, EPO can be produced in the brain, as well as cross the BBB into the systemic circulation to reach peripheral organs. Histological analysis of the hippocampal CA1 region suggests significant differences between the different treatments in this study. The LPS-induced endotoxic shock increased neuronal death (Fig. 1 and 3), as it can reveal the presence of "ghost forms" and the loss of pyramidal cells in this area (Fig. 1). An increase in neuronal death was evident in the LPS groups (Fig. 3). However, when the LPS was administered with EPO (LPS + EPO group), we observed a smaller number of dead cells in the CA1 region, compared with dead cells in the group with endotoxic shock (LPS group). The increase in survival of functional neurons when animals were subjected to endotoxic shock in the presence of EPO could reflect signaling pathways through which EPO prevents apoptotic neuronal death through kinases and anti-apoptotic genes[26,27]. In addition, EPO has the ability to regulate the levels of oxidative stress, which induce the generation of ROS, including superoxide, hydrogen peroxide, oxygen singlet, hydroxyl radical, nitric oxide and peroxynitrites. The EPO limited the generation of these radicals and the extent of cell damage, resulting in a lower rate of neuronal death (Fig. 1 and 3).

Figure 4. Interleukin-1β levels in hippocampal homogenates in the different experimental groups. Data are shown as means ± standard error of the mean of four animals per group. Statistically significant differences were observed between the LPS group and all other groups ($p \geq 0.0001$). There were statistically significant differences between the melatonin (MLT) group and lipopolysaccharide (LPS) groups (LPS + erythropoietin [EPO], LPS + MLT, and LPS + MLT + EPO; $p \geq 0.005$).



Erythropoietin operates at different levels within the CNS, limiting the production of ROS, modulating neurotransmission, preventing apoptosis, and reducing the inflammatory process[28,29]; because of these effects generated by EPO, it is possible to suggest that it has neuroprotective effects.

During endotoxic shock, a large number of cytokines and pro-inflammatory mediators are generated, including TNF-α, IL-1β, and IL-6. Pro-inflammatory cytokines may downregulate the expression of EPO-mRNA, but in turn, they can increase the expression of EPO-R in astrocytes[28]. The EPO attenuates inflammation by reducing reactive astrocytosis and microglial activation, thereby inhibiting the recruitment of immune cells to damage areas[21,28,30]; in cultured cerebrovascular endothelial cells, EPO downregulates the levels of TNF- α induced by the expression of IL-6, as well as the levels of IL-1α, chemokine receptor type 4 (CXCR4), and IL-1β. The EPO directly inhibits the effects of interferon-α, and the alterations through cytotoxicity induced by LPS in oligodendrocytes that affect the integrity of white matter[28]. Furthermore, EPO influences the release of TNF-α and reduces its effects in Schwann cells[28]. In this study, TNF-α and IL-6 were not detected, while the mean levels of IL-1β induced by endotoxic shock (LPS group) were reduced by nearly 72% in the presence of EPO (LPS + EPO group; Fig. 4).

Neuronal activation of the EPO-R to prevent apoptosis induced by N-methyl-D-aspartate (NMDA) receptor or nitric oxide involves the crosstalk of signals between the Janus Kinase-2 (JAK-2) and nuclear factor kappa beta-light chain enhancer of activated-B cells (NFkB) second messenger pathways, the latter being activated by IL-1β[28,29]. The protection provided by the exogenously administered EPO is associated with the prevention of the increase in IL-1β levels and the attenuation of leukocyte infiltration after hypoxia/ischemia-reperfusion injury[31]. In a large variety of nervous system disease models where EPO fulfills a protective role, inflammation is a pathogenic component induced by cytokines and chemokines, and it is followed by leukocyte infiltration or enhanced glial activation[31,32]. The EPO neuroprotection is attributed to a delay in the production and release of pro-inflammatory cytokines by microglia and astrocytes, as well as a significant reduction in the influx of inflammatory cells in the brain parenchyma[31].

We have provided evidence suggesting that endogenous administration of EPO prevents the neuronal death induced by endotoxic shock, as well as diminishes the levels of pro-inflammatory cytokine IL-1β in the rat CA1 region. However, the molecular mechanisms through which exogenous administration of EPO exerts these protective effects are still not fully understood and only some components of the proposed pathways have been identified.

Neuronal cell loss caused by endotoxic shock, as well as IL-1β levels, can be reduced by the administration of the hematopoietic cytokine EPO during the early

stages of shock. Accordingly, EPO may provide important benefits when used to treat sepsis and endotoxic shock.

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PERMANYER

Rev Inves Clin. 2016;68:299-304 ORIGINAL ARTICLE

Advantages of Minimally Invasive Surgery for the Treatment of Colovesical Fistula

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ABSTRACT

Background: Colovesical fistulas in two-thirds of the cases are due to diverticular disease. In recent years, a minimally invasive approach has shown advantages over the traditional open approach. The goal of this study was to evaluate the surgical results and safety of the laparoscopic procedure in patients with colovesical fistula. **Material and methods**: We retrospectively evaluated 24 patients who underwent surgery for colovesical fistula in a referral center from 2005 to 2011. Patients were divided into two groups: (i) laparoscopic approach, and (ii) open approach. **Results**: The laparoscopic and open groups had similar characteristics with respect to age and gender distribution. There were a higher number of bladder repairs in the open approach group (83.3 vs. 16.6%; p = 0.01). The operative time (212 ± 74 min vs. 243 ± 69 min; p = 0.313) and intraoperative bleeding (268 ± 222 ml vs. 327 ± 169 ml; p = 0.465) were similar in both groups. The conversion rate of the laparoscopic approach to open surgery was 25%. There was no difference in morbidity (41.1 vs. 25%; p = 0.414), although the laparoscopic group had a shorter hospital stay (9 ± 4 days vs. 15 ± 11 days; p = 0.083) without statistical significance. **Conclusions**: The treatment of colovesical fistula by a laparoscopic approach is safe and is associated with less bladder repairs and a shorter hospital stay. (REV INVES CLIN. 2016;68:299-304)

Key words: Colovesical fistula. Diverticular disease. Laparoscopy.

INTRODUCTION

Colovesical fistula was described for the first time in 1888. Diverticular disease is the cause of these fistulas in two-thirds of the cases. However, they have also been described in other conditions such as colon or bladder neoplasms, radiation to the pelvis, or Crohn's disease[1,2].

The clinical diagnosis is sometimes difficult because pneumaturia and fecaluria, which are pathognomonic

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signs, are found in late stages and with a low frequency[2,3].

Acute sigmoid diverticulitis is the most common cause of fistula. The incidence of colovesical fistula in diverticular disease is 2-23%. The pathophysiologic mechanism of this condition is the direct extension of a ruptured diverticulum or erosion of a peridiverticular abscess into the bladder. There is always a risk of formation of a fistula between an inflamed diverticulum and an adjacent organ. In addition, fistulas into the cecal appendix, fallopian tubes, uterus, ureters, and skin have been described. The colovesical fistulas are the most common kind, followed by colovaginal fistula in patients who have undergone hysterectomy[4-6].

Traditionally, surgical treatments of colovesical fistulas include resection of the sigmoid colon and fistulous tract, and bladder repair[7]. With the advent of laparoscopic colon surgery, this technique has been found to offer advantages such as lower levels of surgical trauma, postoperative adhesions, pain, and ileus. However, there are few studies evaluating the outcomes of colovesical fistula surgery using a minimally invasive approach[8-12].

The aim of our study was to evaluate postoperative outcomes and length of hospital stay after a minimally invasive approach compared to open surgery in colovesical fistula repair.

MATERIAL AND METHODS

Study subjects

A review of patients who underwent surgery for colovesical fistula from 2005 to 2011 in a reference center was carried out using a prospectively collected database. The colovesical fistula was diagnosed by rectal water-soluble contrast tomography, barium enema, cystography, and/or colonoscopy. The diagnosis was confirmed through surgical findings and histopathology results in all cases. Demographic variables, operative bleeding, surgical time, morbidity, mortality, and length of hospital stay were recorded. Weight was classified according to body mass index (BMI): a BMI < 18.5 was considered malnutrition; BMI 25-30, overweight; and > 30 was defined as obesity[13]. The preoperative albumin serum levels were > or < 3 g/dl. Anemia was defined as a hemoglobin level < 12 g/dl, and diabetes was defined as a serum glucose level > 126 mg/dl[14].

Surgical procedures

The type of surgery to be performed was decided according to the surgeon's preference. All patients received mechanical bowel preparation using polyethylene glycol. Oral antibiotic prophylaxis with erythromycin and neomycin was used one day before surgery, and intravenous antibiotic prophylaxis with cefuroxime was used 30 minutes before surgery. Surgery consisted of resection of the sigmoid colon and a colorectal anastomosis by resecting the fistulous tract. Bladder integrity was assessed by a urology specialist using the methylene blue test. A urinary catheter was placed in all patients whether or not they underwent bladder repair; this catheter was held in place for 10 days after surgery. Colorectal anastomosis was performed by double stapling technique using circular staplers 31 or 29 mm. Laparoscopic procedures were performed using five trocars. In the laparoscopic approach, vascular pedicles were dissected using vascular staplers and/or vessel sealing devices. Colon resection was extended to the rectosigmoid junction. All patients underwent a hydropneumatic test to verify the integrity of the anastomosis.

Statistical analysis

Results are expressed as means with standard deviation. Fisher's exact test was used to analyze nonparametric variables, and Student's *t*-test was used to analyze quantitative variables. Statistical significance was assigned a value of $p < 0.05$. Statistical analysis was performed using the SPSS Software v16 (SPSS Inc. Chicago Illinois, USA).

RESULTS

Twenty-four patients were included, 12 in each group. The average age was 56 years in the laparoscopic and 57 years in the open group; 91% of patients were male in both groups. Comparison of other demographic variables is shown in table 1.

Regarding the surgical procedure, 10 resections with primary anastomosis (83.4%), and two Hartmann's

Table 1. Demographic variables

	Laparoscopic (n = 12)	Open (n = 12)	p
Average age (years)	56 (34-83)	57 (40-81)	NS
≤ 50 years	9 (75%)	9 (75%)	
Male gender	11 (91.6%)	11 (91.6%)	NS
Body weight			
Obesity	3 (25.0%)	2 (16.6%)	0.615
Overweight	3 (25.0%)	6 (50.0%)	0.206
Undernourished	1 (8.3%)	2 (16.6%)	0.539
Albumin			
> 3 mg/dl	9 (75%)	6 (50%)	0.206
≤ 3 mg/dl	3 (25%)	6 (50%)	
Hemoglobin			
> 12 mg/dl	10 (83.3%)	10 (83.3%)	NS
≤ 12 mg/dl	2 (16.6%)	2 (16.6%)	
Glucose			
≥ 126 mg/dl	10 (83.3%)	11 (91.6%)	0.535
< 126 mg/dl	2 (16.6%)	1 (8.3%)	

Table 2. Comparison of intraoperative variables

	Laparoscopic (n = 12)	Open (n = 12)	p
Surgery			
Sigmoidectomy+ PA	10 (83.3%)	10 (83.3%)	NS
HP	2 (16.6%)	2 (16.6%)	
Bladder repair	2 (16.6%)	10 (83.3%)	0.010
Surgical time (min)	212 ± 74.78	243 ± 69.32	0.313
Bleeding (ml)	268 ± 222	327 ± 169	0.465
Transfusion	2 (16.6%)	1 (8.3%)	0.537
Conversion	3 (25%)		

HP: Hartmann's procedure; PA: primary anastomosis.

procedures were performed in both groups. None of the patients who had undergone Hartmann's procedure underwent intestinal reconnection due to the presence of comorbidities in these patients.

Bladder repair was more frequent in patients who had undergone open surgery (83.4 vs. 16.6%; p = 0.01). The surgical time was 212 ± 74.78 min in the laparoscopic group and 243 ± 69.32 min in the open group (p = 0.313).

Intraoperative bleeding was 268 ± 222 ml and 327 ± 169 ml in the laparoscopic and open groups, respectively. Transfusion was required for two patients in the laparoscopic group (16.6%) and one patient in the open group (8.3%). There was a conversion rate of 25% (3/12) because of poor anatomical exposure in two cases and bleeding in one case. These three procedures were completed by open surgery without complications (Table 2).

There were three complications in each group (25%): one anastomosis leak and two urinary tract infections in the laparoscopic group; and one surgical wound infection, one anastomosis leak, and one lesion of the left ureter in the open group. The latter was repaired successfully during the same surgery.

The hospital stay was 9 ± 4 days in the laparoscopic group and 15 ± 11 days in the open group (p = 0.083). Morbidity and hospital stay length were not higher in patients undergoing conversion in the laparoscopic group (11 days).

No recurrences or deaths occurred in either group after a mean follow-up of 18.6 months (7-69 months).

DISCUSSION

Diverticular disease of the colon is a common condition occurring in more than one-third of the population over 45 years old. It is known that only 20% of patients will develop a diverticulitis-associated event at 10 years after diagnosis[6]. To our knowledge, this is the first report on this complication in our country and in Latin America.

Among the first series of laparoscopic treatment for diverticular disease of the colon complicated by the development of fistulas are the studies performed by Kockerling, Bouillot, and Le Moine. Kockerling, et al. included 55 patients with colovesical fistula (18.1% of 304 cases with diverticulitis undergoing laparoscopic surgery) in their case series[10]. They describe that 28.9% of cases with perforated diverticulitis, fistula, or lower gastrointestinal tract bleeding had associated morbidity, versus 14.8% in cases with only peridiverticulitis, stenosis, or undergoing repeat surgery for recurrence; the conversion rate to open surgery was 31.8 vs. 7.2% in elective cases and 18.2% in cases with peridiverticulitis, stenosis, or undergoing repeat surgery for multiple recurrences[10]. Bouillot, et al. included 154 patients (136 cases with intracorporeal anastomosis and 18 cases with manual anastomosis via mini-laparotomy) with a surgical time similar to our series (223 ± 79 min). Mortality was zero and reported morbidity was 14%, with a conversion rate of 13.9%. Unlike our study, their patients undergoing conversion had a longer hospital stay (9.3 vs. 13.0 days)[15]. Eijsbouts, et al. describe a series of 70 cases, four of which underwent surgery by laparoscopic approach and three by "facilitated" laparoscopic approach (splenic flexure and sigmoid mobilization using laparoscopic surgery and dissection of the rectosigmoid junction and the fistulous tract through a Pfannenstiel mini-laparotomy)[16].

Liberman, et al. compared 14 patients with open or laparoscopic approaches, three of whom had colovesical fistula[17]. Although no sub-analysis regarding patients with fistula was conducted, favorable findings in the minimally invasive approach, including intraoperative bleeding (171 vs. 321 ml), start of a liquid diet (2.9 vs. 6.1 days), and hospital stay (6.3 vs. 9.2 days), were observed. Additionally, savings of approximately US$ 12,000 in favor of the laparoscopic approach were found when comparing the total average hospital charges.

In 2005, Bartus, et al. described 40 patients with diverticular fistula who underwent surgery, 36 of whom had laparoscopic surgery with an average hospital stay of 6.2 days shorter when compared with our results, but with surgical times similar to those reported by us (an average of 220 vs. 212 min), and a conversion rate identical to that in our study (25%). In the study by Bartus, et al. they had no anastomotic leaks or repeat surgeries, unlike our study where there were two events[18]. The fundamental importance of that publication is that the development of fistulas secondary to diverticular disease of the colon does not contraindicate curative surgery using a minimally invasive approach.

In our study, primary anastomosis was performed in 83.4% of patients and 16.6% of patients underwent Hartmann's procedure. There is information that supports the construction of a primary anastomosis in these patients. Milesky, et al.[19] described a series of 27 patients where resection and primary anastomosis were performed in 51.8% of cases, resection and Hartmann's procedure in 11.1% of cases, and a derivative colostomy was performed in only 33.3%. The overall hospital stay for each group was 10, 33, and 57 days, respectively. Meanwhile, Walker, et al.[20] described a total of 14 patients, 85.7% of whom were treated with resection and primary anastomosis without morbidity related to dehiscence. Furthermore, there were no recurrences with this approach. Both groups recommend staging treatments for severe inflammatory processes, the presence of large abscesses, or other conditions not associated with diverticular disease (severe post-radiation damage, long-standing traumatic injury, inflammatory bowel disease, or local-regionally advanced cancers). Delaney, et al., in a series of 24 cases of colovesical fistula caused by diverticular disease, reported that 95.8% of the cases were treated by resection and primary anastomosis, with only one case derived by loop ileostomy[21]. Moreover, the conversion rate was subdivided depending on the involved or origin organ (27.4% in sigmoid vs. 15.4% in bladder, vs. 25% in our series). Similar to our findings, they reported no statistically significant differences regarding complication rates, repeat surgeries, and readmission between laparoscopic and converted cases. In a study of 31 patients, Holroyd, et al.[22]

performed sigmoidectomy and anterior resection with protective loop ileostomy in 18.1% of cases. No colorectal anastomosis was protected after the isolated sigmoidectomy.

Menenakos, et al. reported a conversion rate different to that found by us (5.5%)[23]. This is a striking finding because more extensive procedures (sigmoidectomy and extended left colectomy in 22.22%) were performed in their study. However, the authors report a recurrence rate of fistula in 5.5%, probably related to partial/segmental resections of sigmoid in 11.1%. Similarly, the conversion rates reported by Burke, et al.[24] and Royds, et al.[25] for this procedure range from 0 to 36%, similar to that reported in our series.

The morbidity rate reported in the literature ranges from 4 to 46% and mortality, from 0 to 30%[15-25]. In our series, morbidity was 25% and mortality was null.

Regarding intraoperative variables, it is important to note that in our series the surgical time was similar to that reported in the literature. Additionally, there was a trend to a lower surgical time in the laparoscopic group. This may be due to selection bias because the most complex cases were probably performed by celiotomy. Bartus, et al. reported an average surgical time of 50 minutes higher for the minimally invasive approach[18]. In a series of 42 patients, Abbass, et al. showed a trend to lower surgical time for diverticular disease complicated by colovesical fistula compared with elective resections without fistula, without reaching statistical significance (251 vs. 281 min; $p = 0.36$)[26]. In their series, bleeding (150 ml for open and laparoscopic procedure) and the need for transfusion (0% for laparoscopic and 5% for open) were similar in both groups and lower than our results (268 ml for laparoscopic vs. 327 ml for open and 16.6% for laparoscopic vs. 8.3% for open, respectively). We believe that the main difference in surgical time in our series is due to a lower percentage of bladder repairs in the laparoscopic group; such repair was performed routinely in open procedures. Despite the fact that bladder closure was performed less frequently in the laparoscopic group, morbidity was not higher. This is similar to that reported by Ferguson, et al. who did not perform bladder repair in 67%, and there were no differences in recurrence, urinomas, or vesicocutaneous fistula[27]. All of our patients remained with a urinary catheter for 10 days after surgery, and only cases in which leakage of methylene blue was evident during surgery were repaired.

The recurrence rate reported in the literature ranges from 0 to 11% (for cases secondary to diverticular disease of the colon)[18-26,28]. In our series, no patient had recurrence. Lynn, et al. reported a rate of 11%; the patients who underwent complex bladder repair showed higher recurrence rates (6 vs. 24%; $p = 0.022$)[28]. Other risk factors for recurrence include rectal and urethral diseases, presence of malignancy, and history of radiotherapy[28]. We believe that our recurrence rate was zero because all anastomoses were performed in the upper third of the rectum, there was no need to perform complex bladder repairs, and all cases were due to diverticular disease of the colon.

Oberkofler, et al. reported a closure rate of colostomy of 66% for patients with primary anastomosis and 90% for protective loop ileostomy[29]. In our series, 16.4% of patients underwent terminal colostomy, and they were not reconnected due to a high surgical risk.

Abbass, et al. reported an average BMI of 31 in a series of 42 patients[26]. In our series, 50% of patients were overweight or obese. As in their series, our patients underwent sigmoidectomy with colorectal anastomosis.

The average hospital stay reported in other series for the minimally invasive approach ranges from 6 to 12 days[18-26,28,30]. In our series, there was a trend to shorter hospital stay in the laparoscopic group (9 ± 4 days vs. 15 ± 11 days; $p = 0.083$). We believe that a statistical significance was not reached due to the small number of patients and the retrospective nature of the study. In this respect, our study shows no real advantage of the laparoscopic approach.

We found a reduced need for bladder repair in the laparoscopic approach; we believe this may be due to a selection bias in this group due to the retrospective design of the study. When performing the procedure by open surgery, we usually check the fistulous tract in the bladder wall with further debridement of nonviable tissue at the edges, and the defect is closed in two planes. When we perform the surgery by laparoscopy, we repair only the visible defects without drying the major portion of the path, which is not translated

into a greater number of urinary fistulas; we attribute this to the rapid healing of the bladder due to the lack of distention provided by the Foley catheter left for a week. On the other hand, when there was no need for bladder repair, the presence of the fistula could be questioned; however, the diagnosis of colovesical fistula was always confirmed with diagnostic studies and corroborated during surgery.

In conclusion, the treatment of colovesical fistula using the minimally invasive approach offers some advantages such as safety similar to that of the open approach, with a likely shorter hospital stay. In our study, no difference in morbidity was found among patients undergoing bladder repair. Sigmoid resection with anastomosis within the upper third of the rectum seems to offer a lower recurrence rate. We suggest conducting prospective randomized studies to consider the laparoscopic approach as standard treatment for colovesical fistula.

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PERMANYER

Rev Inves Clin. 2016;68:305-13 ORIGINAL ARTICLE

Impact of Aberrant Antigens in the Outcome of Patients with Acute Leukemia at a Referral Institution in Mexico City

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ABSTRACT

Background: Patients with acute leukemia can express aberrant markers, defined as antigens that are normally restricted to a different lineage. The reported significance and frequency of these markers is inconclusive. We assessed the frequency and impact of aberrant markers in patients with acute leukemia in a referral institution in Mexico City. **Methods:** We included 433 patients, diagnosed and treated between 2005 and 2015 in our institution. **Results:** Aberrant markers were expressed in 128 patients (29.6%); CD13 and CD33 were the most frequent aberrant markers in patients with acute lymphoblastic leukemia, while CD7 and CD19 were the most frequent in patients with acute myeloid leukemia. In the univariate analysis, the group with aberrant markers had a lower disease-free survival when compared with the aberrant-free group (8 vs. 13 months) ($p = 0.03$). Aberrant expression of CD10, CD20, and CD33 correlated with a worse outcome in a statistically significant manner. In the multivariate analysis, male gender, lymphoid lineage, secondary leukemia, high risk at diagnosis, and the presence of aberrant markers had a significantly negative impact on disease-free survival. **Conclusion:** The use of more aggressive treatment strategies could be considered in patients with acute leukemia and an aberrant expression of CD10, CD20, and CD33. (REV INVES CLIN. 2016;68:305-13)

Key words: Aberrant marker. Acute leukemia. Acute lymphoblastic leukemia. Acute myeloid leukemia. Flow cytometry.

INTRODUCTION

The adequate classification of acute leukemias (AL) is of paramount importance to provide the patient with the best possible treatment. In the past, ALs were classified based on morphological and cytochemical features. More recently, new classification schemes have arisen based on the implementation of immunophenotypic, cytogenetic, and molecular markers[1]. Flow cytometry performs a fast and comprehensive determination of antigen expression in ALs and, alongside the morphological features of the blast cells, it can

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provide a definitive diagnosis and discriminate between the different types of ALs, which are: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and acute leukemia of ambiguous lineage that includes mixed phenotype acute leukemia (MPAL) and acute undifferentiated leukemia (AUL)[1,2].

In addition to detecting the antigens that allow for the correct classification of ALs, flow cytometry has also aided in the identification of aberrant markers. These are antigens that are normally expressed in a certain lineage, but in some instances are expressed on the blasts of an AL that belongs to another lineage (i.e. CD13 in ALL)[3]. In the medical literature, the reported frequency of this phenomenon is highly variable[3,4]. For example, in one study it was reported that CD13, a myeloid-related antigen, was present in 22% (n = 6/27) of ALL cases, while in other studies the expression of CD13 as an aberrant marker was reported in 53% (n = 10/71) and 37% (n = 46/124) of patients with ALL[5-7]. Moreover, no consensus has been reached about the clinical significance of this phenomenon. Some authors have demonstrated a lower disease-free survival (DFS) and overall survival (OS) in ALL patients when CD13 is present as an aberrant marker[8,9]. Other studies have reported no prognostic impact or a favorable outcome when CD13 is expressed in ALL patients[10,11].

With the aim of expanding the knowledge about this phenomenon, we retrospectively assessed the frequency and prognostic impact of aberrant markers in 433 Mexican patients with AL diagnosed and treated at a referral academic institution in Mexico.

MATERIALS AND METHODS

Patients

All the patients with AL diagnosed and treated between January 2005 and June 2015 at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) were included in this study. The diagnosis of AL and its classification were based initially on the French-American-British (FAB) scheme, and later the classification proposed by the World Health Organization (WHO) in 2008[12,13]. A 20% blast threshold was used for the diagnosis of AL; blast percentages were derived from peripheral blood smears or from blood marrow samples stained with Wright-Giemsa[13].

The following data were gathered from the patients' medical record: date of diagnosis, laboratory results corresponding with the date of diagnosis, the immunophenotypic features of bone marrow or peripheral blood samples, and the performance status of each patient according to the Eastern Cooperative Oncology Group (ECOG) Scale.

By using international criteria (i.e. leukocytes and age at diagnosis, cytogenetics), patients were stratified according to their risk and were divided into non high-risk and high-risk groups[14]. Patients with incomplete data and those who were diagnosed or followed up at another institution were excluded from the study.

The following parameters were recorded: occurrence of complete remission (CR) and relapse, as well as DFS and OS. Complete remission was defined as the presence of < 5% of blasts in the bone marrow aspirate one month after the induction therapy was initiated (+28 days), along with the absence of blasts in peripheral blood, no extramedullary leukemia infiltrations, an absolute neutrophil count ≥ 1×10^9/l, and platelet counts ≥ 100×10^9/l[15,16]. Disease-free survival was defined as the interval between CR and relapse, and OS was defined as the interval between diagnosis and last follow-up date or date of death from any cause.

Lastly, the patients were stratified into two groups: patients without aberrant markers and patients with aberrant markers. We analyzed if there was any difference in the clinical, laboratory, and survival parameters between these two groups.

Treatment

Three main strategies were used in the treatment of patients diagnosed with ALL during this 10-year period: (i) hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (hyper-CVAD) and derived regimens (i.e. hyper-CVAD plus rituximab or imatinib, when appropriate); (ii) local or institutional regimens modified from the German Multicenter Study Group for ALL (GMALL) protocol[17]; and (iii) pediatric-based therapies for adults (i.e. the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01).

Regarding the treatment of patients with AML, we used the typical 7+3 protocols (cytarabine and

doxorubicin/daunorubicin), and the all-trans retinoic acid (ATRA)-based regimens for acute promyelocytic leukemias. Since November 2010, we started to introduce more intensive induction therapies in patients with AML, including the 7+3+7 protocol (adding etoposide) and intermediate or high doses of cytarabine (IDAC and HIDAC, respectively).

Ethics approval

The study was approved by the institutional review boards of ethics and research of our institution.

Flow cytometry

Ethylenediaminetetraacetic acid anticoagulated blood, bone marrow, or both specimens were processed and stained using a pre-lysing technique with a variable combination of the following antibodies: CD2, surface CD3 (sCD3), cytoplasmic CD3 (cCD3), CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD41, CD56, CD61, CD64, cCD79a, CD117, CD235a, anti-MPO, HLA-DR, sIgM, cIgM. An eight-color flow cytometry panel was used, which included the following fluorochromes: phycoerythrin (PE), fluorescein isothiocyanate (FITC), peridin chlorophyll (PerCP), allophycocyanin (APC), V450 and V500, PE and cyanine dye 7 (PE-Cy7), and PerCP-Cy5.5. Data were obtained and analyzed on a FACSCanto™ II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) with the aid of the FACSDIVA™ software (Becton Dickinson, San José, CA, USA). The samples were analyzed using flow cytometry with a CD45 gating technique, which allowed the analysis of only the blast population. The expression of cytoplasmic markers (MPO, CD3, CD79a and IgM) and CD34 was considered positive if these markers were present in 10% or more of the blast population, 20% or more of the blast population for myeloid markers and HLA-DR, and 30% or more for lymphoid markers[18].

Statistical analysis

For categorical parameters, the two-tailed Chi-square and the Fisher exact tests were used for group comparison. For continuous variables, the Mann-Whitney, Kruskal-Wallis, and the median test were used to compare non-normal data between groups. For the survival analysis, the log-rank test was used in the univariate analysis, and the Cox regression test was used for the multivariate analysis. Statistical analysis was performed with the SPSS Software package, v21.0 (SPSS Inc., Chicago, USA).

RESULTS

Clinical and laboratory features

A total of 433 patients with AL were diagnosed and treated at our institution between 2005 and 2015. Acute lymphoblastic leukemia was diagnosed in 216 patients (49.9%), AML in 208 (48%), and MPAL in nine (2.1%). The median follow-up was seven months (range 0-123). Patients were divided into two groups: a group characterized by the presence of aberrant markers and another group without such markers. The clinical and laboratory characteristics at the time of diagnosis are shown in table 1; there were no statistical significant differences between both groups at diagnosis.

Association of aberrant markers with clinical outcomes

Expression profile of aberrant markers

One hundred and twenty-eight (128/433, 29.6%) patients with AL expressed aberrant markers, distributed as follows: 86 patients (19.9%) expressed one marker, 24 (5.5%) expressed two, and nine (2.1%) expressed three or more markers. The other nine patients (2.1%) were diagnosed with MPAL, and their blast cells expressed both myeloid and B-cell markers.

Expression of aberrant markers in acute lymphoblastic leukemia

In the 64 patients (64/216, 29.7%) who were diagnosed with ALL and expressed aberrant markers, these antigens were distributed as follows: 53 (82.8%) expressed myeloid markers, eight B-cell ALL (12.5%) expressed myeloid and T-cell markers simultaneously, two B-cell ALL (3.1%) expressed T-cell markers, and one T-cell ALL (1.6%) expressed a B-cell marker. Of the 57 patients that had B-cell ALL, the most frequent aberrant markers were CD13, CD33, CD15 and CD2. In the seven patients with T-cell ALL, CD13 and CD33 were also the most frequent markers. Detailed data of aberrant markers expression is presented in table 2.

Table 1. Demographic and clinical characteristics of patients

	Without aberrant markers No. patients (%) (n = 305)	With aberrant markers No. patients (%) (n = 128)	p
Gender			0.916
Male	167 (54.8)	71 (55.5)	
Female	138 (45.2)	57 (44.5)	
Age, years			
Median (range)	40 (15-88)	39 (16-85)	0.96
≥ 65	48 (15.7)	18 (14.1)	0.77
Cell lineage			0.517*
ALL	152 (49.8)	64 (50)	
AML	153 (50.2)	55 (43)	
MPAL	-	9 (7)	
Leukemia etiology			0.445
De novo	282 (92.5)	115 (89.8)	
Secondary	23 (7.5)	13 (10.2)	
Risk			0.516
Non-high risk	192 (63)	76 (59.4)	
High risk	113 (37)	52 (40.6)	
ECOG			0.458
0	43 (14.1)	14 (10.9)	
1-2	193 (63.3)	73 (57)	
3-4	8 (2.6)	3 (2.3)	
Hemoglobin, g/dl			0.964
Median (range)	8.19 (3.4-15.4)	8.3 (4.8-14.6)	
Leukocyte count, $\times 10^9$/l			0.52
Median (range)	7.5 (0.1-422.9)	5.75 (0.2-337.0)	
Platelet count, $\times 10^9$/l			0.665
Median (range)	38 (5-1,123)	33 (0-472)	
Blasts, %			
PB, median (range)	44.5 (1-98)	62 (5-100)	0.763
BM, median (range)	48 (1-98)	61.6 (2-94)	0.788

* Fisher's exact test was used for the comparison of the presence of antigen markers between acute myeloid leukemia and acute lymphoblastic leukemia only.
ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BM: bone marrow; ECOG: Eastern Cooperative Oncology Group; MPAL: mixed-phenotype acute leukemia; PB: peripheral blood.

Expression of aberrant markers in acute myeloid leukemia

Of the 55 patients (55/208, 26.4%) who were diagnosed with AML and expressed lymphoid markers, 31 (56.4%) expressed T-cell markers, 19 (34.5%) expressed B-cell markers, and five (9.1%) expressed both T-cell and B-cell markers. The most frequent lymphoid markers in AML were CD7, CD19, CD2, and CD22.

Association of aberrant markers with complete remission and relapse rates

Of the 433 patients with AL, 242 (55.9%) achieved CR, 140 (32.3%) relapsed at least once, and 293 patients (67.7%) died. The differences between the groups with and without aberrant markers are shown in table 3. The only statistically significant difference between the two groups was the number of deaths: 64.6% in the aberrant-free group vs. 75% in the individuals with aberrant markers (p = 0.042).

Association of aberrant markers with overall and disease-free survival

In the univariate analysis, the median OS of the group without aberrant markers was 12 months (range 0-114) vs. eight months (0.07-123) in the group with aberrant markers (p = 0.06); median DFS in the group without markers was 13 months (range 0-109) vs. eight months (0-84) in the group with aberrant markers (p = 0.03) (Fig. 1). In the univariate analysis, the variables that had a negative impact on OS were: age

Table 2. Expression of aberrant markers in acute leukemia

Aberrant Marker	AML No. patients (%) (n = 55)	B-cell ALL No. patients (%) (n = 57)	T-cell ALL No. patients (%) (n = 7)
CD2	9 (16.4)	6 (10.5)	–
CD5	1 (1.8)	1 (1.7)	–
CD7	21 (38.2)	5 (8.8)	–
CD10	3 (5.5)	–	–
CD11b	–	5 (8.8)	2 (28.6)
CD13	–	37 (64.9)	6 (85.7)
CD14	–	1 (1.7)	0
CD15	–	15 (26.3)	2 (28.6)
CD19	11 (20.0)	–	0
CD20	1 (1.8)	–	0
CD22	8 (14.5)	–	0
CD33	–	18 (31.6)	3 (42.8)
CD41a	–	1 (1.7)	0
CD56	6 (10.9)	1 (1.7)	–
CD64	–	1 (1.7)	0
CD79a	6 (10.9)	–	1 (14.3)
CD117	–	5 (8.8)	2 (28.6)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia.

at diagnosis (≥ 65 years), etiology of AL (secondary leukemia), risk at diagnosis (high risk), initial ECOG (≥ 3), and lack of CR (Table 4).

For DFS, the variables that negatively affected this clinical endpoint were: gender (male), cell lineage (lymphoid), etiology of AL (secondary), risk at diagnosis (high risk), and, as stated before, the presence of aberrant markers. In the multivariate analysis, which included all of the variables that had a statistically significant impact, only DFS was affected in a statistically significant manner by the presence of aberrant markers (Table 4).

Table 3. Complete remission, relapse, and overall and disease-free survival rates

	Without aberrant markers No. patients (%) (n = 305)	With aberrant markers No. patients (%) (n = 128)	p
Complete remission	170 (55.7)	72 (56.3)	1.000
Relapse	95 (31.1)	45 (35.2)	0.432
Deceased	197 (64.6)	96 (75.0)	0.042

When comparing the number of aberrant markers on the leukemic blast population, no statistically significant association was observed between the patients that had one, two, or three or more aberrant antigens and DFS or OS. However, in patients with three or more aberrant markers, there was a trend towards a shorter DFS and OS (p = 0.06 and p = 0.059, respectively).

Association of specific aberrant markers with overall and disease-free survival

The individual analysis of specific aberrant markers showed a lower OS in patients with AML that expressed CD20 (nine months in CD20-negative vs.

Figure 1. Association of aberrant markers with overall survival **(A)** and disease-free survival **(B)** (log-rank test).

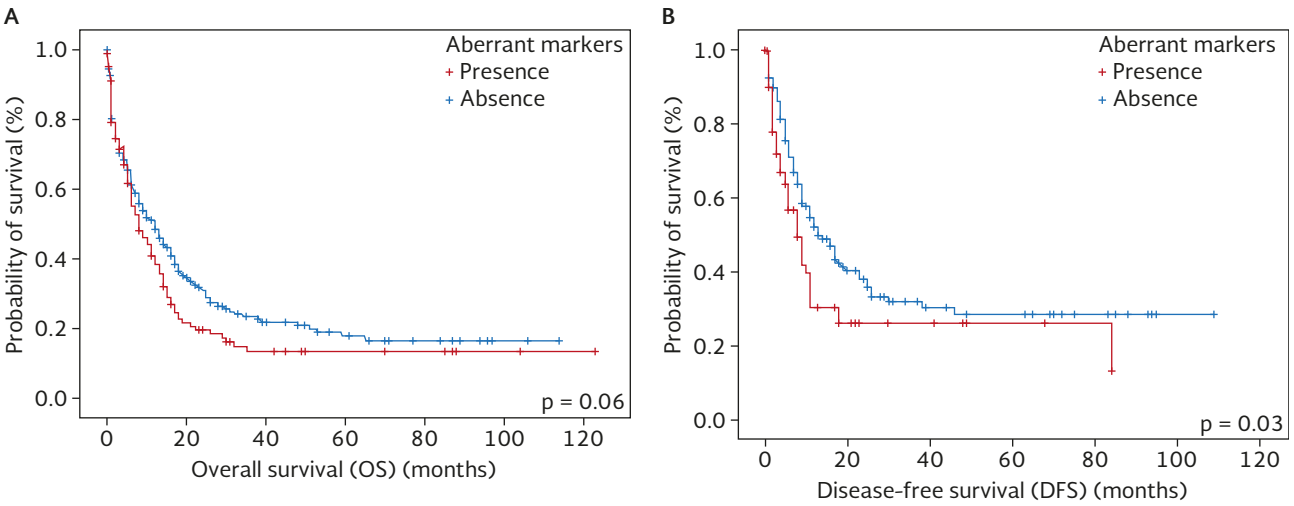

Table 4. Univariate and multivariate analysis of factors influencing overall survival

Variables	OS, months	Univariate	Multivariate	
	Median (range)	p	HR (95% CI)	p
Age, years		< 0.001	0.79 (0.548-1.444)	0.213
< 65	13 (0-123)			
≥ 65	3 (0-28)			
Gender		0.170		
Lineage		0.163		
Etiology		< 0.001	1.058 (0.673-1.665)	0.806
De novo	12 (0-123)			
Secondary	5 (0.23-30)			
Risk		0.042	1.098 (0.836-1.441)	0.502
Non-high risk	12 (0-114)			
High risk	8 (0-123)			
ECOG		< 0.001	1.447 (1.204-1.704)	< 0.001
0	17 (0-84)			
1-2	10 (0-54)			
3-4	8 (0-12)			
Aberrant markers		0.06		
Complete remission		< 0.001	0.134 (0.096-0.188)	< 0.001
Not achieved	2 (0-48)			
Achieved	19 (1-123)			

ECOG: Eastern Cooperative Oncology Group; OS: overall survival.

0.13 months in CD20-positive) (p < 0.001) and in patients with ALL that expressed CD33 (13 months in CD33-negative vs. six months in CD33-positive) (p = 0.01). Additionally, DFS was shorter in the patients with AML that expressed CD10 (18 months in CD10-negative vs. two months in CD10-positive) (p = 0.001) (Fig. 2).

Other associations

We also analyzed the adjusted prognostic value of the aberrant antigens in the non high-risk and high-risk groups. For OS, we found no significant differences in the median survival between the non high-risk group and the high-risk group (p = 0.066) (Table 4). Regarding DFS, we found a statistically significant difference between the non high-risk group and the high-risk group (p = 0.047) (Table 5).

Impact of the treatment regimens

Of the 216 patients diagnosed with ALL, 196 (90.7%) received a proper induction therapy, divided as follows: 112 patients (57.1%) received the hyper-CVAD or derived regimens, 56 (28.6%) received local or institutional regimens, 11 (5.6%) were treated with pediatric based protocols, and 17 patients (8.7%) received other induction protocols. When adjusting the prognostic value of the aberrant antigens between the different treatments used, we found no significant differences in OS and DFS in the patients treated with hyper-CVAD or derived regimens. On the other hand, DFS was significantly different between patients that received the local or institutional regimens (p = 0.044) (Table 6).

Regarding the patients with AML, 137 (65.9%) received an induction therapy as follows: 81 (59.1%) received a typical 7+3 protocol, 17 (12.4%) received an intensive induction protocol (either 7+3+7 or IDAC/HIDAC), and 18 patients (13.1%) received other induction protocols. The remaining 21 patients (15.4%) had acute promyelocytic leukemia and all received an ATRA-based regimen. When adjusting the prognostic value of the aberrant antigens between the different treatments used, we found no significant differences in OS or DFS.

DISCUSSION

In our study, aberrant antigens were expressed in 27.5% of patients with AL (27.8% of ALL and 26.4% of AML patients; p = 0.517). In both, B-cell and T-cell

Figure 2. Aberrant expression of CD20 in acute myeloid leukemia **(A)** and CD33 in acute lymphoblastic leukemia **(B)** and their association with overall survival; and CD10 in acute myeloid leukemia, **(C)** and its association with disease-free survival (log-rank test).

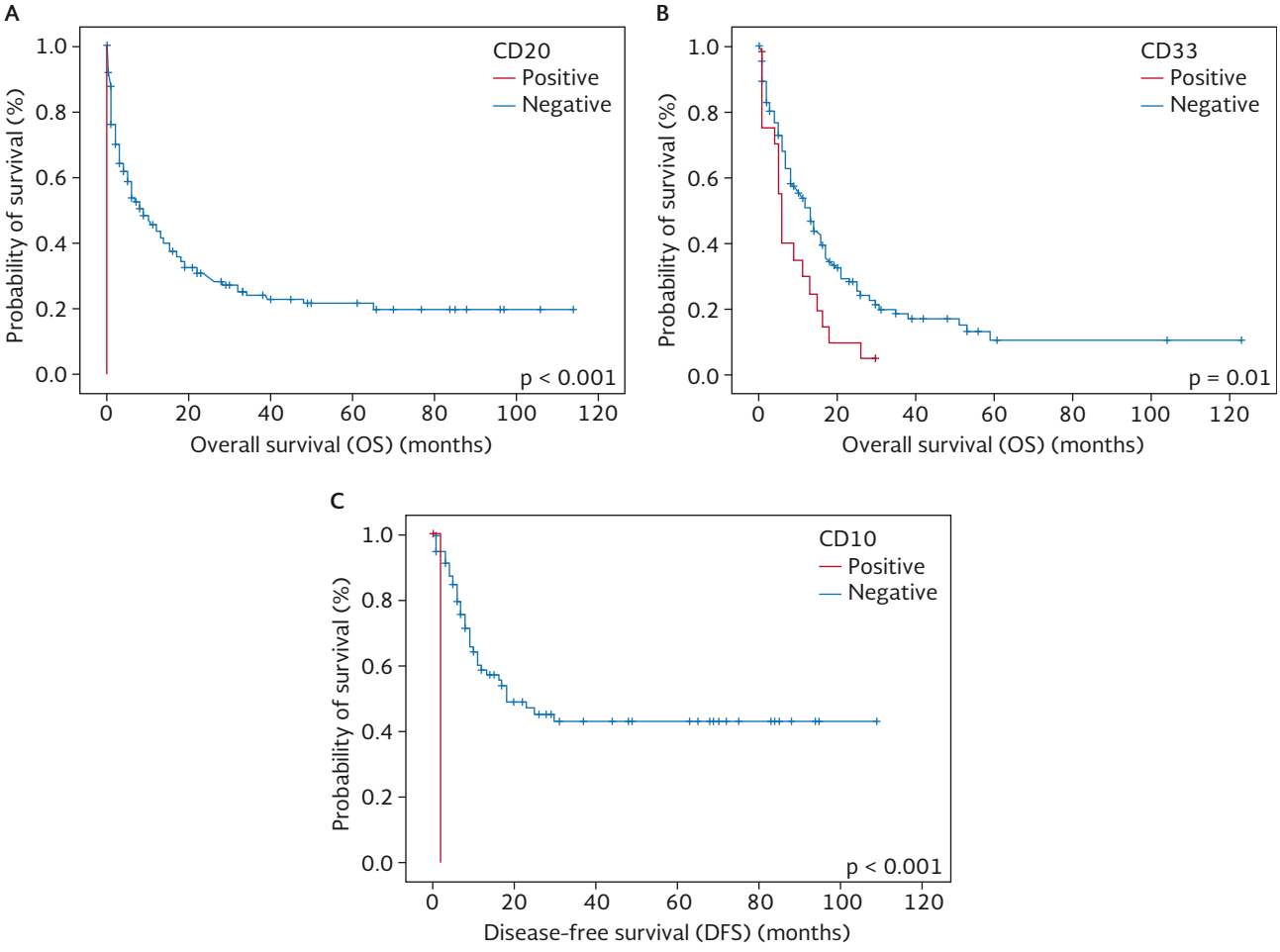

Table 5. Univariate and multivariate analysis of factors influencing disease-free survival

Variables	DFS, months	Univariate	Multivariate	
	Median (range)	p	HR (95% CI)	p
Age		0.647		
Gender		0.025	0.678 (0.483-0.952)	0.025
Male	9 (0-94)			
Female	17 (0-109)			
Lineage		< 0.001	0.911 (0.637-1.305)	0.612
Lymphoid	9 (0-93)			
Myeloid	18 (0-109)			
Etiology		0.065		
Risk		< 0.001	2.452 (1.694-3.551)	< 0.001
Non-high risk	17 (0-109)			
High risk	6 (0-84)			
ECOG		0.179		
Aberrant markers		0.03	1.488 (1.040-2.127)	0.03
Absence	13 (0-109)			
Presence	8 (0-84)			

DFS: disease-free survival; ECOG: Eastern Cooperative Oncology Group.

Table 6. Prognostic value of aberrant markers adjusted to risk at diagnosis and treatment protocols

Variables	OS, months Median (range)	p	DFS, months Median (range)	p
Risk at diagnosis		0.066		0.047
Non-high risk without aberrant markers	13 (0-114)		23 (0-109)	
Non-high risk with aberrant markers	11 (0.67-88.0)		11 (0-84)	
High risk without aberrant markers	11 (0-65)		8 (0-63)	
High risk with aberrant markers	7 (0.13-123.0)		3 (0-84)	
ALL protocols				
Hyper-CVAD regimens without aberrant markers	14 (1-71)	0.656	12 (0-70)	0.578
Hyper-CVAD regimens with aberrant markers	15 (67-104)		11 (0-84)	
Institutional regimens without aberrant markers	16 (1-94)	0.234	11 (0-91)	0.044
Institutional regimens with aberrant markers	6 (0.4-123.0)		3 (0-41)	
AML protocols				
Intensive regimens without aberrant markers	25 (1-38)	0.501	30 (0-37)	0.538
Intensive regimens with aberrant markers	19 (14-50)		11 (3-49)	
7+3 without aberrant markers	13 (0.2-106.0)	0.655	13 (0-95)	0.446
7+3 with aberrant markers	6 (0.46-88.0)		11 (0-84)	

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; DFS: disease-free survival; Hyper-CVAD: hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone; OS: overall survival; 7+3: cytarabine and doxorubicin/daunorubicin.

ALL, CD13 and CD33 were the most common aberrant markers, and in patients with AML the most common were CD7, CD19, CD2 and CD22. As stated before, no consensus exists on the frequency of aberrant markers, as exemplified by the lack of agreement in several studies[19-26].

A statistically significant association has been reported between the presence of myeloid markers in adult ALL and a lower CR rate, as well as a shorter survival[27]. In our study, in addition to a higher number of deaths, patients with aberrant markers had an inferior DFS when compared with the aberrant-free individuals in both the univariate and multivariate analyses. It is important to state that in the multivariate analysis, the inferior DFS was statistically significant in spite of the presence of certain factors that, in the univariate analysis, predicted a worse outcome. Furthermore, a trend was observed in our results regarding a lower DFS when three or more aberrant markers were present.

The presence of CD33 as an aberrant marker in patients with ALL has been related with a poor prognosis or with a lack of any clinical impact[28]. Other researchers have shown an association between the presence of CD7, CD56, and CD79 as aberrant markers and a poor outcome in patients with AML[29,30]. In the present study, AML patients expressing CD20 and ALL patients expressing CD33 as aberrant markers achieved a shorter OS than the survival attained by the aberrant-free group. Additionally, AML patients had a shorter DFS when CD10 was expressed as an aberrant marker.

One of the advantages of this study is the inclusion of a rather large adult population with AL expressing aberrant markers. Furthermore, when comparing the prognostic value of the aberrant markers adjusted by the risk at diagnosis and by the use of the different induction chemotherapies, we found that patients who presented aberrant markers and were categorized as high risk at diagnosis, or were treated with local or institutional regimens, had a worse median DFS than those with the same characteristics but without aberrant markers. Therefore, we can state that the use of hyper-CVAD should be preferred over the local or institutional regimens when treating patients with ALL who present aberrant markers. Moreover, based on this research, the use of aggressive treatment strategies (i.e. as in high-risk individuals) could be suggested in the aforementioned patients, as well as AML patients expressing CD10 and CD20 and ALL patients expressing CD33[31,32].

Based on the fact that the literature is inconclusive about the significance and frequency of this phenomenon, and considering that this study had some limitations due to its retrospective nature and to the use of rather heterogeneous induction regimens, we cannot

suggest the immediate translation of our results into everyday clinical practice. Thus, to reach a consensus on the meaning and frequency of this phenomenon, we suggest that future research could follow these recommendations: (i) uniformity in the threshold for positivity of the various antigens; (ii) standardization of the monoclonal antibodies used to detect these antigens; (iii) use of similar populations in a given study; and (iv) use of similar chemotherapeutic regimens, which might affect the outcome of patients with AL when interpreting the prognostic significance of an aberrant marker[33].

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PERMANYER

Rev Inves Clin. 2016;68:314-8 ORIGINAL ARTICLE

Usefulness of Gum Chewing to Decrease Postoperative Ileus in Colorectal Surgery with Primary Anastomosis: A Randomized Controlled Trial

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ABSTRACT

Background: Postoperative ileus generates a high impact on morbidity, hospital stay, and costs. **Objective:** To study the efficiency and safety of chewing gum to decrease postoperative ileus in colorectal surgery. **Method:** A randomized controlled trial was performed including 64 patients who underwent elective colorectal surgery with primary anastomosis in a tertiary referral center. Patients were divided in two groups: (i) A: gum chewing group (n = 32), and (ii) B: patients who had standard postoperative recovery (n = 32). **Results:** Postoperative ileus was observed in 6% (2/32) of the gum-chewing group and in 21.8% (7/32) in the standard postoperative recovery group, with an odds ratio of 0.167 (95% CI: 0.37-0.75; p = 0.006). Vomiting was present in two patients from group A and in eight from group B (6.25 vs. 25.0%; p = 0.03). Passage of flatus within the first 48 hours was present in 30 patients from group A and in 20 from group B (94 vs. 63%; p = 0.002). There was earlier oral feeding (96 ± 53 vs. 117 ± 65 hours; p= 0.164) and a shorter length of hospital stay (7 ± 5 vs. 9 ± 5 days; p= 0.26) in the gum-chewing group (p N.S.). **Conclusions:** The use of chewing gum after colorectal surgery was associated with less postoperative ileus and vomiting, and with an increased passage of flatus within the first 48 hours after surgery. Since gum chewing is an inexpensive procedure and is not associated with higher morbidity, it can be safely used for a faster postoperative recovery in elective colorectal surgery. (REV INVES CLIN. 2016;68:314-8)

Key words: Postoperative ileus. Chewing gum. Colorectal surgery. Postoperative recovery.

INTRODUCTION

Postoperative ileus (POI) is a state of gastrointestinal transit arrest secondary to an abdominal surgical procedure. It is characterized by abdominal distention, absent bowel sounds, and inability to tolerate oral intake[1-3]. POI is considered a common issue in colorectal surgery, with a prevalence of up to 15%[4]. Due to its high frequency, POI may impact on morbidity and hospital stay, with increased costs[4,5].

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The etiology of POI is multifactorial. The exposure of the peritoneal cavity and intestinal manipulation provokes systemic and local inflammatory responses in the intestinal muscular wall due to sympathetic activation in response to surgical stress. This favors the action of proinflammatory mediators, such as vasoactive intestinal peptide, neurotensin, and nitric oxide, and an increase in leukocytes levels at the surgical site, promoting gastrointestinal hypomotility[6-8]. Gastric motility usually recovers approximately 24-48 hours after an abdominal surgical procedure; however, the colon has a slower recovery pattern, ranging from 48 to 72 hours[9,10].

Several studies have shown that inhibiting the inflammatory response reduces postoperative ileus. Some of the procedures that have been used to reduce the POI include regional blocks, early mobilization, early oral feeding, avoiding nasogastric tubes, minimally invasive surgery, less intestinal handling, normothermia, prokinetic drugs, and nutrition with lipid-rich supplements. It has been suggested that opioid analgesics used in the perioperative period reduce the time required to restore normal intestinal motility[2,4,11].

Sham feeding is a procedure where food and drink are not actually digested, but a regional cephalic-vagal response is activated, promoting an increase in plasma gastrin levels, substance P, and pancreatic polypeptide. Gum chewing has shown this same response[4,12,13].

Several studies have evaluated the usefulness of chewing gum in colorectal surgery, showing discordant results. In two controlled studies, the benefit of chewing gum to reduce POI was not established [14,15]; however, a meta-analysis showed that the passage of the first flatus, time for the first evacuation, and hospital stay were all reduced[16].

The aim of the present was to analyze the benefits and security gum chewing in patients undergoing elective colorectal resection with primary anastomosis.

MATERIALS AND METHODS

This was a prospective randomized trial that included 64 patients who underwent elective colorectal surgery in a tertiary referral center during the period from July 2010 to December 2011. The study was reviewed and approved by the ethical committee of the hospital, and written informed consent was obtained for all patients. Our local Ethical Committee does not require registering clinical trials in foreign databases.

All patients older than 18 years old, operated for benign or malignant colonic diseases, were electively included. Exclusion criteria were emergency surgery, patients requiring a stoma, and those admitted to the intensive care unit. Certified colorectal surgeons performed all surgeries. Postoperative analgesia was performed with acetaminophen and ketorolac in all patients; neither prokinetic drugs nor opioid analgesics were used after surgery. Ciprofloxacin (400 mg) was administered intravenously to both groups 30 minutes before the skin incision; an additional dose was given every three hours during the surgery. After the surgery, no additional antibiotics were given to the patients. No antiemetic drugs were used in any patient.

Diet was initiated when each patient had had passage of flatus and peristalsis was present. The progression of the diet was gradually changed from liquids during 24 hours to solids, and was continued until the discharge of the patient. Surgical residents and the nurses in charge supervised chewing gum administration and data was registered in a prospective database.

Patients were divided in two groups: (i) A: gum-chewing group, and (ii) B: patients who had standard postoperative recovery. Group A patients began to chew sorbitol-free gum within the first 24 hours after surgery, for 15 minutes every four hours throughout their hospital stay, with six resting hours at night. Nurses of each patient supervised the chewing gum administration protocol, whenever it was indicated. Patients from group B received no further intervention. Both groups were compared in terms of serum albumin levels, hemoglobin, ASA scale (American Society of Anesthesiology), and comorbidities. The primary endpoints were the presence of ileus, time for the first flatus, and hospital stay. The presence of nausea, vomiting, abdominal distension, oral tolerance at 72 hours, postoperative complications, and mortality were also analyzed. Patients were deemed to have POI when they had absence of adequate bowel function on postoperative day 5, or the need for the insertion of a nasogastric tube because of abdominal distension, nausea, and emesis after having started a liquid diet, in the absence of mechanical obstruction[17]. The passage of

flatus was recorded by directly asking the patients, and it was measured in hours from the day of surgery until its appearence. Flatus passage at 48 hours was dichotomously reported as positive or negative. Abdominal distension was subjectively recorded by asking the patients whether the symptom was present or not. The results were analyzed according to the group assigned.

Anastomotic leaks were defined by the presence of signs of peritoneal irritation and were corroborated by computed tomography scan of the abdomen with contrast or by surgical findings. The hospital stay was defined as the number of days in the hospital including the admission day until patient discharge. Patients were discharged when they tolerated oral feeding, and evacuation was achieved. Surgical site infections were defined according to the guidelines issued by the Centers for Disease Control and Prevention[18]. A research assistant performed the group allocation with sealed envelopes.

Continuous variables are expressed as means plus/minus standard deviation (SD) and were assessed using the student *t* test. Categorical variables were analyzed using Chi-square test or Fisher's exact test, as appropriate. All tests were two-tailed, and a value of $p< 0.05$ was considered statistically significant. The sample size was calculated with the statistical software G*Power 3.1. Based on a previous study[13], the time to the first bowel movement was estimated to be 3.7 days in the gum group and 4.5 days in the other group, with a SD of 2.4 in both groups. It was determined on the basis of power (80%) and a two-sided α of 0.05. Thus, 32 patients were required in each group. We did not create models for multivariate analysis to evaluate independent variables since patient numbers were too small for parameters identified by the univariate analyses. The analysis was performed using SPSS v21.0 for Windows (SPSS, Chicago, IL).

RESULTS

Sixty-four patients were included, 32 in each group. The mean age was 56 and 50 years in groups A and B, respectively (18-96 years). The male to female ratio was 1.46. Comparisons between groups are shown in table 1.

Table 1. Comparison between groups

Variables	Group A (n = 32)	Group B (n = 32)
Albumin (g/dl)	3.4 ± 0.47	3.5 ± 0.5*
Hemoglobin (g/dl)	13.2 ± 1.4	13.1 ± 1.4*
ASA 1/2/3/4	16/16/0/0	16/15/1/0*
Comorbidities (Y/N)	3/29	2/30*
Surgical procedures		
Right colectomy	13	16
Sigmoid colectomy	12	12
Left Hemicolectomy	5	2
Total colectomy	2	2

*p = NS. Standard deviation = ±.
ASA: American Society of Anesthesiology.

Surgical indications for group A were colon cancer in 22 patients, diverticular disease in six, one stenosis of colorectal anastomosis, one recto-vaginal fistula, one Crohn's disease, and one Hartmann reversal procedure. Surgical indications for group B were colon cancer in 17 patients, diverticular disease in eight, two colocutaneous fistulas, two colovesical fistulas, one appendicular mucocele, one lymphoma, and one chronic colonic variceal bleeding. The types of surgery are summarized in table 1. Fifty percent of the patients in each group were operated by minimally invasive surgery. Stapled anastomoses were used in 91% and 94% in groups A and B, respectively (p = 0.64). There was no difference in surgical bleeding between groups (Group A = 162 ml ± 169 vs. Group B = 223 ml ± 217; p = 0.21).

Postoperative ileus was confirmed in two patients of group A and in seven of group B (6.0 vs. 21.8%; p = 0.006), with an odds ratio of 0.167 (95% CI: 0.37-0.75). Nausea was reported in six patients from group A and 11 from group B (18.7 vs. 34.3%; p = 0.15). Vomiting was present in two patients from group A and in eight from group B (6.2 vs. 25.0%; p = 0.03). Bloating was reported in four and seven patients in groups A and B, respectively (12.5 vs. 21.8%; p = 0.32). Passage of first flatus within the first 48 hours was present in 30 patients of the chewing gum group and in 20 from the other group (94 vs. 63%; p=0.002).

Oral feeding within the first 72 hours was initiated in 27 patients from group A, and 21 from group B (84.4 vs. 65.6%; p= 0.08). There was earlier oral feeding (96 ± 53 vs. 117 ± 65 hours; p = 0.164) and a shorter length of hospital stay (7 ± 5 vs. 9 ± 5 days; p = 0.26)

Table 2. Overall outcomes

Variables	Group A		Group B		P
	n = 32	%	n = 32	%	
Postoperative ileus	2	6	7	22	0.006
Nausea	6	19	11	34	0.15
Vomiting	2	6	8	25	0.03
Abdominal bloating	4	13	7	22	0.32
Flatus passage at 48 hours	30	94	20	63	0.002
Oral feeding before 72 hours	27	84	21	66	0.08
Hospital stay (days)	7.5 ± 5.1		9 ± 5.7		0.26

Table 3. Postoperative complications

Postoperative complications	Group A	Group B*
Pneumonia	3 (9.3%)	2 (6.2%)
Surgical site infections		
Superficial incisional	5 (16%)	5 (16%)
Organ or space		
With anastomotic leakage	1 (3.1%)	1 (3.1%)**
Without anastomotic leakage	2 (6.2%)	3 (9.3%)***

*Overall complication rates: 34.3 vs. 34.3%; p = 0.58.
**Required re-laparotomy. ***Required radiological intervention.

in the gum-chewing group, without statistical significance for the differences observed. Overall results are shown in table 2. Overall morbidity in each group was 34%.

There was one anastomosis leakage in each group (3.1%). Surgical site infections were 16% in both groups (Table 3). Mortality was nil.

DISCUSSION

Postoperative ileus is defined as the physiological arrest of gastrointestinal transit in response to surgical stress. The POI arises from autonomic nervous and hormonal mechanisms. Its origin is multifactorial as operating time, intestinal manipulation, inflammatory response, administration of opioids, or anxiolytic medications play a role in this physiological response[11,12,19].

A meta-analysis of 158 patients reported a reduction in the time of passage of flatus (20.8 hours), bowel movements (33 hours) and hospital stay (2.4 days) in patients receiving gum for elective colorectal surgery, with a trend to lower postoperative complications (OR: 0.45; 95% CI: 0.20-1.00; p = 0.05)[16]. Similar to this study, we reported more flatus passage in the first 48 hours and a trend towards shorter hospital stays (7 ± 5 vs. 9 ± 5 days; p= 0.26) in patients who chewed gum. In another meta-analysis of 272 patients, it was observed that the use of gum decreased POI after gastrointestinal surgery, without a significant reduction in the hospital stay[20]. The authors conclude that the results are not significant in gastrointestinal laparoscopic surgery. However, we found a lower POI and shorter length of hospital stay in patients who chewed gum, considering that half of our patients were operated by minimally invasive surgery.

Lim, et al. [4] reported a clinical trial of 161 patients, where patients who chewed gum and were subjected to a fast-track program in colorectal surgery showed no differences in the mean time of passage of first flatus and bowel movements, with similar percentages of nausea (77.5 vs. 83.1%), vomiting (43.8 vs. 46.8%) and abdominal bloating (72.5 vs 70.1%) compared to controls. In contrast, our study showed that the gum-chewing group had lower vomiting events and a trend toward lower nausea and abdominal bloating.

In another prospective study, Van Den Heijkant, et al.[2] found a trend toward shorter hospital stay (9.5 vs. 14 days) and decreased POI (27 vs. 48%). This study found that the levels of interleukin 8 and tumor necrosis factor were lower in patients who chewed gum, who also presented lower postoperative complications. In our study, we found lower POI with the same percentage of postoperative complications in both groups (34%). The presence of POI in our study was similar to that reported globally in colorectal surgery (17.4 %)[1].

In another meta-analysis that included 244 patients, Vazquez, et al.[11] reported a decrease in time for passage of flatus and for first fecal evacuation, without differences in hospital stay (p = 0.10).

A British meta-analysis with 256 patients showed favorable results when chewing gum for a prompt passage of flatus and decreased time for fecal evacuation, with no significant differences in hospital stay[21].

Yiu, et al.[22] evaluated the use of chewing gum in another meta-analysis of 612 patients, from which 271 patients belonged to enhanced recovery after surgery protocols (ERAS). In this study, a significant decrease of 31 minutes was seen for the passage of the first flatus, 30 minutes for the passage of stool, and a shorter hospital stay; however, when evaluating the subgroup of patients included in the ERAS group, no significant difference was found when chewing gum was used additionally[22].

Finally, in a Cochrane review of 81 studies with 9,072 patients undergoing abdominal surgery [23], the subgroup of patients who underwent colorectal surgery was analyzed. This review found favorable results in gum chewing for a prompt recovery of gastrointestinal motility. The results showed a decrease of 12.5 hours for the start of flatus, 18.1 hours for the first bowel movement, and a slight decrease of hospital stay (one day). There was no difference in mortality, risk of infections, and readmissions among groups and the gum was well tolerated by patients. In our study, we found that patients in the gum group had less vomiting episodes and passed more flatus at 48 hours, with lower POI.

Based on our results, we wanted to compute our achieved statistical power *post hoc* with POI (6 vs. 22%). Given a type 1 error of 0.05, with a sample size of 32 patients, our achieved power (1-β error probability) resulted in 0.85 or 85%.

In conclusion, the use of chewing gum after colorectal surgery was associated with less POI and vomiting, with an increased passage of flatus within the first 48 hours after surgery. Since it is an inexpensive tool and is not associated with higher morbidity, it can be safely used for a faster postoperative recovery in elective colorectal surgery.

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