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Sialochemical analysis in polytraumatized patients in intensive care units

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Abstract

The profiles of polytraumatized patients in intensive care units were characterized. Serum and salivary markers were compared with normality between Classes I and II of APACHE II and between periods of hospitalization; these results were correlated. This was a prospective study on saliva charts and collection (n = 70). Profile: male, 27 years old, blunt traumas and collisions. Serum parameters with normality: decrease in pH, creatinine at admission to Class I, and at 48 and 72 hours in both classes; K^+ at 48 h in Class II; Ca+ on admission in both classes and at 72 h in Class I. Increase in urea at 72 h in Class II, glucose at all times and in all classes, and Ca+ at 48 h in both classes. Class II had high Na^+ at 48 and 72 h compared to Class I. In Class I, creatinine reduction occurred in 48 h and 72 h compared to admission and an increase of Ca+ at 48 h with admission. In Class II, pH and Na^+ increased at 48 h and 72 h compared to admission. K^+ decreased from admission to 48 h and increased from 48 h to 72 h. Urea increased from 48 to 72 hours. Creatinine decreased from admission to 48 and 72 hours. Ca+ increased from admission to 48 hours and decreased from 48 to 72 hours. There was an increase in the saliva levels in both classes and times in relation to normality. There was an increase in urea at admission, glucose at 72 h, and Ca+ at 48 h in Class II compared with Class I. Class I urea increased from admission to 48 h and Ca+ decreased from admission to 48 h. Class II urea decreased from 48 h to 72 h. Strong or very strong positive correlation was identified between blood and creatinine saliva at all times and regular and negative Ca+ at 72 h. This study provides evidence that salivary and serum biomarkers can be used together to monitor the evolution of the clinical symptoms of ICU patients.

Introduction

According to the World Health Organization (WHO), 5.8 million people die each year from trauma worldwide, surpassing by 32% the deaths caused by malaria, AIDS and tuberculosis combined, which corresponds to 10% of all causes of death. Without due intervention, it is anticipated that this incidence will increase until 2030, dramatically raising personal and social costs. Traumas also account for a majority of permanent disabilities and are responsible for considerable economic losses to victims, their families, and the countries in general [1–3].

A trauma is defined as "one or more lesions, of varying extent, intensity and severity, which may be produced by various agents (physical, chemical, electrical), either accidentally or intentionally, capable of producing local or systemic disturbances" [2–5]. A consequence of trauma is polytraumatism, which is classified according to the Trauma Committee of the American College of Surgeons—ATLS (*Advanced Trauma Life Support*), as being "in two or more organ systems, being necessary that a combination of these lesions represent a vital risk to the patient" [5].

In Brazil, this scenario is likely to be repeated, due to the uncontrolled growth of cities, as well as the marginalization of its population, and disparities in social conditions and lifestyles, which characterize it as a conflict-ridden society with a large amount of violence and traffic accidents. This represents a public health problem, with trauma being a relevant consequence [3,6].

In view of this worldwide scenario, the health institutions and their professionals have as a venue for treating these events the intensive care units (ICUs), which are intended for the treatment of recoverable patients at risk of death who need continuous and specialized care [1,7,8]. The need for ICU hospitalization is multifactorial. ICUs are intended for cases of medium and high complexity. Intensive care is aimed at assisting the individual in a severe state in an integral manner, with human resources, materials, and high technology equipment available [9–11]. Treatment and hospitalization in ICUs have contributed to greater survival of patients with the most diverse diseases, due to the development of hard, light-hard, and light technologies. In addition, the need to keep up with daily personal and work activities imposes on the daily lives of citizens a greater health risk and a greater number of long-term complications due to exposure to stressors, among them an increase in the number of circulating vehicles, culminating with higher rates of health damage mainly from accidents involving automotive vehicles. This leads to increasingly complex services and raises costs [8,11].

Treatment costs (including rehabilitation and accident investigation), as well as reduction or loss of productivity, make this process long and costly for health institutions and society as a whole. In addition, studies show that trauma victims are usually in the age range of 5–44 years, most of them men who are in their most productive phase, including economically [1,2]. According to the American Trauma Committee, the estimate for 2020 is that one in ten of these individuals will die from trauma [6].

In ICUs, the control of these patients is done through physical examination performed by the multiprofessional team that works in this environment and image and laboratory examination. The laboratory procedures most used for diagnostic purposes involve analysis of the chemical and cellular constituents of the blood. The most requested blood elements are sodium, potassium, calcium, phosphorus, magnesium, serum creatinine and urea, prothrombin time (PT), activated partial thromboplastin time (APTT), lactic acid, arterial blood gas, capillary glycemia, fasting glucose, blood count, and platelets. However, other biological constituents are also used for these purposes, such as urine, cerebrospinal fluid, feces, and saliva [12,13].

Conventional methods for blood serum analysis involve invasive procedures, and these cause pain and may present health risks, due either to the imperfection of the collector or the poor health condition of the individual in addition to the high cost [14–16]. Given this context, studies have verified the use of saliva for the clinical control of patients with systemic or localized diseases in the mouth, since it can be easily collected non-invasively when compared to blood collection [17,18].

According to Kaufman et al., (2002) [17] the use of saliva for diagnostic tests in patients with systemic diseases has been used for offering distinct advantages over blood. It can be collected non-invasively by individuals with technical training. In addition, saliva can provide a cost-effective approach for tracking large populations. Total saliva (as in the evaluated article), however, is most often used for the diagnosis of systemic diseases, as it contains serum constituents. Therefore, the authors point out that saliva analysis can be useful for the diagnosis of hereditary and autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as for the assessment of therapeutic drug levels and the monitoring of illicit drug use.

Saliva is a biological fluid secreted continuously by salivary glands. Its inorganic part is composed of ions and mineral salts such as chloride, bicarbonate, chlorine, phosphate, iodide, bromide, fluoride, sodium, potassium, and calcium, and the organic part is made up of proteins and enzymes. Its main function is the protection of the buccal tissues as well as other physico-chemical and biochemical properties that aid in speech, food processing and dental protection, and remineralization [17–19]. Studies indicate that the composition of saliva reflects the tissue levels of therapeutic drugs, hormones, and immunological molecules [17]. Studies have revealed the effectiveness of salivary analysis as a substitute for serum analysis in patients with diabetes mellitus (DM) [20] and chronic renal disease (CKD) [21], in children and adolescents [22], and in patients with acute traumatic brain injury ICU [23]. In these individuals hospitalized in an ICU, an increase in cortisol and salivary amylase was observed in children and adolescents (surgical, trauma, oncology), and these results were correlated with increased severity [22]. In adults, there was an increase in cortisol [23]. As far as we know, these are the only studies performed with patients hospitalized in ICU.

In the oral cavity, saliva from the ducts mixes with other secreted products present there, such as oral epithelial desquamative cells, nasal cavity mucus, food debris, microorganisms, organic and inorganic compounds, bacterial metabolism products, mucosal transudate and exudate of gingival grooves; the mix is then called total saliva [17,18]. Salivary flow directly affects the composition of saliva and can be obtained in two ways: unstimulated saliva and stimulated saliva. Unstimulated saliva may correspond to half of the saliva flow stimulated in non-complex patients, and the amount of saliva available is directly linked to oral homeostasis [16,18,19].

When salivary markers (urea, creatinine, glucose, and calcium) were first evaluated in polytrauma individuals in the ICU, the use of total saliva was chosen. Previous studies have shown that saliva has reliable biological properties for analysis of the composition of the oral microbiota, even when there are alterations in the salivary flow, which can be caused by variations in the circadian cycle and factors such as stress and exercises and other systemic alterations, situations present in the ICU [20,22,23,24].

In addition to the serum parameters used to evaluate critical individuals, other tools have been used to subsidize and predict the prognosis of these individuals, such as the Acute Physiology and Chronic Health Evaluation *(APACHE) [25–27]*. This index is a point-based assessment system that emerged from the need to classify groups of patients admitted to ICUs. APACHE is based on the severity of the disease and the estimation of the risk of death through standardized information [26,27]. It was based on the hypothesis that the severity of the acute disease can be quantified by the degree of abnormality of physiological variables. Age and the presence of chronic diseases prior to admission to the ICU were also considered to decrease the physiological reserve and, therefore, directly reflect the patient's survival [26,27]. These variables, considered influential for patient survival, were selected for APACHE [27].

The APACHE system was developed, modified, and validated over three decades of studies: APACHE [25], APACHE II [26], APACHE III [27] and APACHE IV [28]. Among all APACHE systems, APACHE II has been widely used in Brazil and worldwide [28,29]. The Brazilian Ministry of Health, in its ruling number 3.432 of 2010, considered the existence of several indices but recommended that APACHE II be used in all ICUs because it is consecrated by use [28]. In 2010, with the publication of the Resolution of the Collegiate Board of Directors (RDC) No. 7, it recommended that IP APACHE II be used in all ICUs because it is a system of disease severity classification recommended in the specialized scientific literature [30].

IP—APACHE II (Fig 1) is considered easy to apply. The clinical and laboratory variables it uses are routinely collected during intensive care and validated for a wide range of diagnoses [25,26,30,31].

Goals

Due to the lack of studies, to our knowledge, that evaluate the salivary parameters in polytraumatized patients in the general ICU, the possibility of salivary parameters being potential indicators of clinical prognosis, along with blood, the ease and noninvasive way of obtaining saliva and the possibility of doing so, and the possible correlation between serum and salivary parameters, this study is justified.

In view of the above, our objectives were:

a. To characterize the sociodemographic profile and type of lesions in polytraumatized patients hospitalized in the ICUs of a university hospital in the city of Curitiba, Paraná;

b. To compare blood serum levels of pH, sodium, potassium, urea, creatinine, glycemia, and calcium with parameters of normality in polytraumatized patients hospitalized in ICU, stratified according to APACHE II, from 0 to 19 and greater than 20;

c. To verify if there were variations in these levels between the periods of 0, 48, and 72 hours of ICU stay;

d. To compare the salivary levels of urea, creatinine, glucose, and calcium with parameters of normality in polytraumatized patients hospitalized in ICUs, stratified according to APACHE II, from 0 to 19 and greater than 20;

e. To verify if there were variations in these levels between the periods of 0, 48 and 72 hours of ICU stay; and

f. To correlate the salivary and blood parameters of these patients.

Materials and methods

Study design

This was a prospective observational study of a quantitative nature, in which the same patients were evaluated at admission, 48 hours, and 72 hours.

Ethical procedures

Ethical and legal aspects were considered, since the study was conducted in accordance with Resolution 466/12 of the National Health Commission. The project was approved by the Ethics

a) ACUTE PHYSIOLOGICAL SCORE - APACHE II									
PHYSIOLOGICAL SCORE	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperture (C)	>41	39-40,9		38,5-38,9	36-38,4	34-35,9	32-33,9	30-31,9	< 29,9
Mean arterial pressure (mmHg)	>160	139-159	110-129		70-109		50-69		< 40
Heart rate (bpm)	>180	140-179	110-139		70-109	55-69	40-54	< 39	
Respiratory rate	>50	35-49	25-34	12-24	10-11	6-9		< 5	
AaDO2 or PaO2 (depending on FiO2)									
a) FiO2 > 0,5 A-aDO2	>500	350-499	200-349		< 200				
b) FiO2< 0,5 PaO2					>70	61-70		55-60	< 55
pH Arterial	>7,7	7,6-7,69		7,5-7,59	7,33-7,49		7,25-7,32	7,15-7,24	< 7,15
Sodium (serum) (mEq/L)	> 80	160-179	155-159	150-154	130-149		120-129	111-119	< 110
Potassium (serum) (mEq/L)	> 7	6-6,9		5,5-5,9	3,5-5,4	3-3,4	2,5-2,9		< 2,5
Creatinine sérum (mg/dL)	> 3,5	2-3,4	1,5-1,9		0,6-1,4		< 0,6		
Hematocrit (%)	> 60		50-50,9	6-49,9	30-45,9		20-29,9		< 20
White blood cell count	> 40		20-39,9	5-19,9	3-14,9		1-2,9		< 1
Glasgow coma Scale									
SCORE									
Seric Bicarbonate (mEq/L) (use if not collect blood gas)	> 52	41-51,9		32-40,9	22-31,9		18-21,9	15-17,9	< 15

b) points for age					
Points	0	2	3	5	6
age	< 44	45-54	55-64	65-74	> 75
C) Points for chronic illness					
These were measured during the first 24 hours after admission, and utilized in addition to information about previous health status (recent surgery, history of severe organ insufficiency, immunocompromised state) and baseline demographics such as age. The calculation method is optimized for paper schemas, by using integer values and reducing the number of options so that data fits on a single-sheet paper form.					
a) For non-surgical or emergency postoperative patients : 5 ponts					
b) For elective postoperative patients : 2 ponts					
ESCORE APACHE II = A + B + C					

Instrumento APACHE II – Knaus et al, 1985

Fig 1. Lethality rate predicted by APACHE II. Source: Knaus WA, Draper EA, Wagner DP, Zimmerman JE. (1985).

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and Research Committee of the Pontifical Catholic University of Paraná (1,358,439; CAAE: 51055515.3.0000.0020). The research was authorized by the local Research Ethics Committee (no. 1.358.439), the Technical Department of Cajuru University Hospital (HUC), PR, and the Intensive Care Service of the HUC, PR (S1, S2 and S3 Text).

Location of the study

The study was conducted in the ICUs of a university hospital administered by the Paranaense Association of Culture. Currently, the hospital is part of the Marista Group Health Area. It is located in the city of Curitiba, Paraná. These units were chosen because they are among the largest urgency and emergency hospitals in the state and, since 2006, have been accredited by the Ministry of Health as a high complexity unit in orthopedics, traumatology and renal transplants.

Sample selection

The study sample consisted of seventy polytraumatized patients admitted to the hospital who needed hospitalization in the ICU from April 2016 to October 2017. During that period, 857 patients were consecutively admitted to the ICUs. Of these, 180 patients were included in the inclusion criteria because they had available test results.

The inclusion criteria were: between 15 and 65 years of age, polytrauma patients hospitalized at ICUs, with a period of hospitalization longer than 24 hours and up to 72 hours (when the last collection of blood and saliva was performed), intubated with oxygen therapy support, Glasgow Coma Scale (GCS) less than or equal to eight (< or = 8), or Ramsey Scale 5 or 6, regardless of gender. 180 patients met the inclusion criteria.

As exclusion criteria: septic patients at the time of hospitalization, rehospitalization, individuals in brain death at admission and during the collection period, inability to obtain the consent term from their legal representative, unidentified individuals, clinical individuals, known prior co-morbidities, pregnant women, and individuals presenting active bleeding of oral mucosa as well as those with suspected oral infection or known oral infection and insufficient amount of saliva collected. In this way, 28 patients had charts with incomplete data; 5 evolved to encephalic death within 48 hours of hospitalization; 5 family members did not agree to participate in the research; 23 clinical patients were admitted; 22 patients came from the internment ward; 18 patients had sepsis; 9 presented with severe bleeding in the oral cavity.

Data collection

Data referring to the general characterization of the patient were collected through a structured interview and from the medical records. Due to non-authorization by the (unconscious) patient, the researcher approached direct relatives or those responsible for hospitalization at the time of admission to request permission to collect data. Those who agreed to take part in the study signed an Informed Consent Form (S4 Text). The IP APACHE II variables were collected according to the original proposal (S5, S6, S7 and S8 Text) (APACHE II groups), on a form filled out by the ICU medical team 24 hours after the patient's hospitalization. The data collected were the following: sex, age, marital status, schooling, remunerated activity, type of accident, accident period, trauma types, and blood transfusion (S9 Text). To perform the predictive gravity analysis of the 70 patients, we adapted the original 1985 Knaus Scale, for only the two following classes: I: APACHE II: 0 to 19 and II: APACHE II: 20 to 71 [25,26].

Serum collection and analysis

Serum blood results were collected from medical records. The blood collection method followed the routine collection of blood from the ICUs, performed by the nurses from radial and/or femoral artery puncture, shortly after receiving the patient in the ICU. The other samples (48 and 72 hours) were collected directly using the PAM device (in the radial and femoral artery), between 4 and 6 o'clock in the morning, according to the laboratory routine of the service. A mean of ten to fifteen mL were withdrawn in a syringe and were then deposited in disposable gel vacuum tubes (BD-Becton Driver, Franklin Lakes, New Jersey) without anticoagulant. The biochemical analysis of the blood was carried out in the Cajuru-HUC laboratory of clinical analysis (Curitiba, PR, Brazil), and the levels of sodium, potassium, urea, creatinine, calcium, and glucose were quantified using the automatic biochemistry system Integra 400 (Roche, Japan). The pH value was automatically quantified by the Cobas b121 blood gas analyzer (Roche, Switzerland).

Salivary collection and analysis

The total saliva samples were collected by the researcher (MHMC); the sample was calibrated by a specialist (STL) and obtained at 3 different moments: at admission, and at 48 and 72 hours after admission. The procedure was performed by positioning a suction probe 14 or 16, adapted to an airway secretion collecting bottle (bronchus, CAMAHE, Curitiba, Paraná), to a mechanical aspirator. The tip of the catheter was moved in the lingual floor and lingual face of the premolars, being moved from the premolar on one side to the other and passing through the lingual floor in the region of premolars, canines, and central and lateral incisors. The movement was repeated for five uninterrupted minutes. The collected saliva was stored in an airway secretion-collecting vial that was attached to the aspirator. After the collection of saliva, the bottles were hermetically sealed, placed in a thermal container, and sent for freezing at (-) 60˚C (Consul 415 freezer, Curitiba, PR) to the biochemistry laboratory, located at the School of Life Sciences of PUC PR. Biochemical analyses of saliva were performed by an analyst (MHMC), with colorimetric tests from Labtest Diagnostic (Labtest, Lagoa Santa, Minas Gerais). Urea, creatinine, glucose, and calcium were tested. Values were expressed as mg/dL.

For the standardization of the collection technique, we performed a pilot study on five patients.

Statistical analysis

The data were analyzed in a database developed in the IBM STATISTICS SPSS 25.0 program, (SPSS Inc, Chicago, Illinois). An exploratory descriptive analysis of the variables (median, standard deviation, 95% confidence interval for the mean, minimum, and maximum) was performed for each of the categories of the APACHE II variable, in order to compare the findings with the variation range of the gold standard, as described in the literature.

Serum analysis

The Kolmogorov-Smirnov normality test was performed for serum pH, sodium, potassium, urea, creatinine, glucose, and calcium at admission, 48 hours, and 72 hours, according to each category of independent variables considered (Apache II, TBI, facial trauma, spine trauma, chest trauma, abdomen trauma, trauma of an extremity). Since the dependent variables did not present a normal distribution in at least one of the categories of the independent variables, the comparison between the independent variables for two categories at a given time was made using the non-parametric Mann-Whitney U test. The comparison between the times

(Admission x 48, Admission x 72, and 48 x 72) was made using the non-parametric Wilcoxon test for paired samples for each category of the APACHE II independent variable. For the verification of the correlations of two non-parametric variables, the Spearman test was used, with 0.00–0.30 considered weak; 0.30–0.60 moderate, 0.60–0.90 strong, and 0.90–1.00 very strong. The level of significance adopted in all tests was 5%.

Salivary analysis

The Kolmogorov-Smirnov normality test was performed for the calcium variable at admission, calcium 48 h and calcium 72 h. The sample size was 25 in the pairing of calcium at admission x 48 h calcium, and n = 24 in the pairing of calcium 48 h x calcium 72 hours. The test showed that the calcium at admission variable was not normal; the opposite was observed for 48 h calcium and 72 h calcium. Thus, the option was made to choose the non-parametric Wilcoxon test for paired samples to compare calcium at admission x calcium 48 h, and Student's t test for paired samples to compare calcium 48 h x calcium 72 h. For the other variables, since the dependent variables did not present a normal distribution in at least one of the categories of the independent variables, the comparison within each time between the independent variables for two categories was made using the non-parametric Mann-Whitney U test.

For each category of the APACHE II independent variable, the comparison between times (admission x 48, admission x 72, and 48 x 72) was made using the non-parametric Wilcoxon test for paired samples. For the verification of the correlations of the two non-parametric variables, the Spearman test was used, considering 0.00–0.30 weak, 0.30–0.60 moderate, 0.60–0.90 strong, and 0.90–1.00 very strong. The level of significance adopted in all tests was 5%.

Results

Of the patients in this study, 61 (87%) were males and 9 (13%) were females. The mean age was 27 years (39%). Regarding marital status, 28 (40%) were unmarried, 38 (54%) were married, and 3 (4%) were widowed. In relation to schooling, 39 (56%) had incomplete high school. As for reported monthly income, 64 (91%) were engaged in a paid activity. 26 (41%) received values ranging from less than or equal to one minimum wage, and 38 (59%) from two to four times the minimum wage (Table 1).

Regarding the type of trauma, blunt trauma was present in 56 (80%) and penetrating trauma in 14 (20%) patients. Of the blunt traumas (contusions), collisions involving motor vehicles account for 37 cases (53%), 28 (40%) of which involving cars and 9 (13%), motorcycles. Among the penetrating traumas, firearm injuries and white-weapon injuries (Table 2) stood out.

In comparison with the normality parameter, a decrease was observed for: pH at admission in both classes; creatinine on admission to Class I and at 48 and 72 hours for both classes; K^+ in 48 hours in Class II; on admission in both classes and in 72 hours in Class I. An increase in the value of the parameters: urea 72 hours in Class II, glucose at all times and classes, calcium 48 h for both classes.

Comparing the classes, Class II individuals presented higher values of Na^+ at 48 and 72 hours when compared with Class I subjects.

Comparing the times among the Class I subjects, there was a reduction in creatinine at times 48 and 72 hours in relation to admission and an increase in calcium 48 hours when compared to admission.

Comparing the times in the Class II subjects, pH and Na^+ increased at 48 and 72 hours in relation to admission. K^+ decreased between admission and 48 hours and increased between 48 and 72 hours. Urea increased from 48 to 72 hours. Creatinine decreased from admission to

Table 1. Distribution of polytrauma patients admitted to the ICUs of a university hospital, with regard to socio-demographic data.

Sociodemographic variables	Nº	59%
Sex		
Male	61	87%
Female	9	13%
Age group (years)		
15–29	27	39%
30–50	35	50%
51 e +	8	11%
Marital status		
Not married	28	40%
Married	39	56%
Widowed	3	4%
Schooling		
Incomplete fundamental	10	14%
Complete Fundamental	12	17%
Incomplete Secondary	39	56%
Complete Secondary	9	13%
Paid activity		
No	6	9%
Yes	64	91%
Remuneration (x the minimum wage)		
< or = 1	26	41%
2 to 4	38	59%
5 or +	-	-

Data collected in Curitiba, Paraná, Brazil, 2017–2018.

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48 and 72 hours. Calcium increased from admission to 48 hours and decreased from 48 to 72 hours (Table 3).

Regarding normality, all parameters evaluated increased in both classes and in the three times. There was an increase in urea at admission, glucose at 72 h, and calcium at 48 h in Class II individuals, compared to Class I. Among Class I subjects, urea showed an increase between admission and 48 hours, and calcium decreased between admission and 48 hours. As for Class II, there was a decrease in urea between 48 and 72 hours (Table 4).

There was a positive correlation between serum and salivary parameters: in creatinine, very strong at admission and strong at 48 and 72 hours, and in regular calcium in 72 hours (Table 5).

Discussion

This is the first prospective observational study that has investigated the epidemiological profile, serum, and salivary levels in polytrauma patients hospitalized in ICU, to have observed a positive association between the severity of the patient's condition and variation in these metabolites.

The patients' profile was similar to that found in studies with polytraumatized patients in the ICU, with a majority of male patients, young individuals, inserted in the labor market and economically active [1–3]. One hypothesis for this profile would be that adolescents and young adults are more exposed to accidents and other violence, mainly due to inexperience, search for emotions, pleasure in experiencing sensations of risk, impulsivity, and abuse of alcohol or drugs [32]. Regarding the types of accidents, the highest percentage was found to be victims of traffic accidents, which are mainly responsible for the injuries, corroborating the findings of previous studies [33,34]. It is known that over the past 10 years, more than one million people have been disabled due to mechanical traumas in the world, with traffic accidents being mainly responsible for these rates [33–35]. It is worth noting that hospitalization for trauma resulting from a traffic accident may correspond to more than 40% of all ICU hospitalizations, depending on the hospital [7,9]. This represents high hospital costs, material losses, social security costs, and great suffering for the victims and their relatives [36–38].

Regarding the serum parameters evaluated, it was noted that blood acts as a buffer solution, which prevents its pH from undergoing major changes. The pH of blood and extracellular fluids remains in the range of 7.35 to 7.45 [39–41]. The organism supports pH changes ranging from 6.8 to 7.8. Decreased blood pH is called acidemia or acidosis; increased pH of blood, alkalemia or alkalosis [40]. Polytraumatic situations culminate in functional and physiological alterations, mainly due to the involvement of one or more vital systems [5,39], as observed among the individuals of this study. If this ratio is altered in any way, it can cause serious damage to the body with profound metabolic changes, which can cause death [5,39,40]. Patients with polytraumas tend to present alterations in the respiratory pattern (either by direct trauma in the thoracic region or by stress), and with this, the amount of HCO3- (aq) increases greatly in relation to H2CO3 (aq); thus, the pH of the blood rises, triggering an alkaline disturbance. This is due to a very rapid breathing, which decreases the amount of CO2 in the body, shifting the chemical balance to the left and decreasing the amount of H+ (aq) (hence the pH increases) [39]. In addition to polytraumatism, other factors also trigger hyperventilation and alkalosis in individuals, such as: drug use, hyperthermia, excessive exercise, cirrhosis, aspirin overdose, and lung diseases [10,40,41].

Severe acid-base balance alterations are potentially critical, especially when they develop rapidly (a feature very similar to those found in the study population), because they are "polytraumatized". Such abnormalities may directly cause several organic dysfunctions, such as cerebral edema, fractures, decreased myocardial contractility, pulmonary vasoconstriction, and systemic vasodilation, among others [42,43]. Acidosis and alkalosis (respiratory, metabolic, or mixed) are common and clinically significant phenomena in poly-trauma patients in the ICU [42–44].

In this study, the pH value underwent a left shift in the two APACHE II classes at admission. This is justified mainly by the individual having suffered a trauma and by the degree of

Table 2. Distribution of patients according to the type of accident that caused the trauma.

Type of accident	nº	59%
Contusive Trauma		
Collision	28	40
Aggression	9	13
Running over	9	13
Falls	9	13
Electric shock	1	1
Penetrating trauma		
Firearm	9	13
White-weapon	5	7

Data collected in Curitiba, Paraná, Brazil, 2017–2018.

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Table 3. Results of APACHE II and serum markers (PH, Potassium, Sodium, Urea, Creatinine, Glucose and Calcium) of 70 polytraumatized individuals in three times after admission to HUC ICUs, Curitiba, Paraná.

Variable	APACHE II	N	Medium	95% confidence interval for mean		APACHE II	N	Medium	95% confidence interval for mean		Golden pattern
				Lower limit	Upper limit				Lower limit	Upper limit	
CLASS I						CLASS II					
Number of lesions	**0–19**	**11**	2.00A	1.90	3.00	**20–71**	**59**	2.00	2.13	2.60	NA
pH—Admission			7.30Aa	7.31	7.40			7.36Aa	7.32	7.37	**7.35–7.45**
pH—48 hours			7.41Aa	7.38	7.43			7.42Ab	7.39	7.42	
pH—72 hours			7.40Aa	7.37	7.44			7.42Ab	7.37	7.42	
K^+ - Admission			3.90Aa	3.63	4.67			4.10Aa	3.89	4.25	**3.5–5.1 mEq/L**
K^+ - 48 hours			3.90Aa	3.60	3.94			3.50Ab	3.44	3.76	
K^+ - 72 hours			3.90Aa	3.66	4.05			3.70Ac	3.63	4.04	
Na^+ - Admission			139.00Aa	138.27	141.61			139.00Aa	138.71	141.30	**136 – 145mEq/L**
Na+ - 48 hours			137.00Aa	135.81	140.73			142.00Bb	141.16	143.75	
Na+ - 72 hours			137.00Aa	135.75	140.07			143.00Bb	142.12	145.28	
Urea Admission			33.00Aa	22.96	38.30			33.00Aa	31.35	51.09	**16.6–48.5mg/dL**
Urea—48 hours			24.00Aa	19.94	36.77			32.00Aab	33.80	48.43	
Urea—76 hours			33.00Aa	24.47	41.16			40.00Aac	40.94	59.18	
Creatinine—Admission			0.62Aa	0.50	1.02			0.70Aa	0.71	0.96	**0.70–1.20 mg/dL**
Creatinine—48 hours			0.51Ab	0.42	0.73			0.45Ab	0.46	0.89	
Creatinine—72 hours			0.51Ab	0.42	0.75			0.48Ab	0.45	1.01	
Glucose—Admission			151.00Aa	119.44	169.46			148.00Aa	146.45	172.42	**70–115 mg/dL**
Glucose—48 hours			143.00Aa	121.94	169.32			1444.00Aa	139.65	167.53	
Glucose—72 hours			144.00Aa	112.80	157.19			148.00Aa	139.20	156.85	
Calcium—Admission			1.15Aa	1.05	1.32			1.11Aa	1.03	1.38	**1.16–1.32 mg/dL**
Calcium—48 hours			1.70Ab	1.25	1.67			1.45Ab	1.36	1.52	
Calcium—72 hours			1.17Aab	1.09	1.31			1.16Ac	1.15	1.24	

pH = potential Hydrogen ion; Na^+ = sodium; K^+ = potassium.

A-z = APACHE II: 0–19; B = APACHE II: 20 to 71.

Mann-Whitney test = Distinct capital letters indicate differences between classes.

Wilcoxon Test = Distinct lowercase letter reveals differences between times in each class.

NA = does not apply.

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stress by means of gravity, characteristic in this study. After 48 and 72 hours, a normalization of this element occurred, and the possible reason for this is that all patients were on mechanical ventilation (MV) from admission and treatments instituted in the ICU, thus maintaining their progressive control of the initial condition [40–42]. MV is a therapy applied in several clinical situations, depending on the severity and risk of death of patients suffering from polytrauma, in order to adequately maintain the levels of O2 (oxygen) and CO2 (carbon dioxide) gases, indispensable for the maintenance of vital organs. Increasingly, the ICU team uses these technologies with different resources for the control and analysis of respiratory parameters supplied by the ventilator, which will guide the teams to the clinical decisions that these patients undergo [42].

To maintain pH within limits compatible with vital processes, the body has a series of regulatory mechanisms, which are: buffer system (instantaneous), between the respiratory (minutes) and the renal (hours to days) [40,41]. It is emphasized that pH variation leads to frequent and clinically significant disorders in severe ICU patients regardless of etiology (polytrauma, sepsis, or shock) [39–41]. Previous studies have shown that polytrauma patients have a higher

Table 4. Salivary levels of urea, creatinine, glucose, and calcium at admission, 48 h and 72 h, in Classes I and II of APACHE II, and normality standard.

Variable	APACHE II	N	Median mg / dL	95% confidence interval for mean		APACHE II	N	Median mg / Dl	95% confidence interval for mean		Gold standard
				Lower limit	Upper limit				Lower limit	Upper limit	
CLASS I						**CLASS II**					
Urea—Admission *	**0–19**	**11**	24.00Aa	16.41	36.86	**20–71**	**59**	53.00Ba	57.14	91.73	**20 mg/dL**
Urea—48 hours*			49.00Ab	19.05	118.15			54.00Aab	69.87	110.76	
Urea—72 hours *			31.00Aab	17.09	77.91			40.00Aac	48.01	79.20	
Creatinine—admission *			0.72Aa	0.54	1.15			0.69Aa	0.75	1.00	**0.12–0.16 mg/dL**
Creatinine—48 hours *			0.78Aa	0.66	0.93			0.56Aa	0.60	1.21	
Creatinine—72 hours *			0.62Aa	0.47	0.96			0.59Aa	0.67	1.23	
Glucose Admission *			103.21Aa	28.98	17.30			109.46Aa	71.27	124.64	**5.6–18.4 mg/dL**
Glucose 48 hours *			111.64Aa	46.75	196.23			108.52Aa	78.08	132.15	
Glucose 72 hours *			21.59Aa	-116.99	56.68			103.13Ba	72.46	125.91	
Calcium—Admission *			49.35Aa	29.41	53.66			33.45Aa	28.73	38.46	**5–7 mg/dL**
Calcium—48 hours **			12.61Ab	6.23	33.73			32.07Ba	26.77	37.03	
Calcium—72 hours **			17.43Aab	12.68	43.62			30.69Aa	26.07	34.76	

UTIs of HUC Curitiba, Paraná. A = APACHE II: 0–19; B = APACHE II: 20 to 71.

Mann-Whitney test = Distinct capital letters indicate differences between classes.

*Wilcoxon test

** Student's t test = Distinct lowercase letter reveals differences between times in each class.

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risk of developing severe metabolic abnormalities, especially acute metabolic acidosis, which is associated with an increase of 0.6 mEq/L in the serum potassium concentration for each 0.1 decline in pH [39,40,43].

Sodium and potassium electrolytes are important for maintaining this equilibrium. In APACHE Class II individuals, K^+ was normal at admission, decreased at 48 hours compared to the gold standard, and returned to normal at 72 hours. Class II subjects presented with higher values of Na^+ at 48 and 72 hours when compared to Class I individuals but within the parameters of normality. Although the results obtained show little variation in relation to normality, it is worth mentioning that sodium and potassium are essential components of body fluids, such as blood, saliva, and urine, helping to regulate the distribution of water throughout the body and playing a fundamental role in basic acid balance. Each and every discomfort to the body, characteristic of polytraumatized individuals, affecting target organs such as the kidneys, heart, and liver, culminates in a risk to the regulation of the volume and composition of body fluids as well as the maintenance of electrolyte balance [39,43,44].

In addition to the above, it is worth mentioning that variation in serum sodium concentration is an important determinant of blood osmolarity, since hyponatremia and hypernatremia are associated with severe brain disorders (cerebral edema), a situation that is very possible in polytraumatized patients [44]. The possible variations between Na^+ and K^+ reinforce the fact that in these polytraumatized individuals there is greater difficulty in maintaining normal osmolality because they tend to retain Na^+ and water in the extracellular medium and increase the concentration of K^+ in the intracellular medium over time, due to the long period of

Table 5. Spearman's correlation coefficient between serum and salivary parameters.

Spearman Correlation Coefficient	Saliva Urea on Admission	Saliva Urea 48 hours	Saliva Urea 72 hours	Saliva Creatinine in Admission	Saliva Creatinine 48 hours	Saliva Creatinine 72 hours	Saliva Glucose on Admission	Saliva Glucose 48 hours	Saliva Glucose 72 hours	Saliva Calcium on Admission	Saliva Calcium 48 hours	Saliva Calcium 72 hours
Blood Urea on Admission	0.065											
Blood Urea 48 hours		0.103										
Blood Urea 72 hours			- 0,025									
Blood Creatinine on Admission				.954*								
Blood Creatinine 48 hours					.735*							
Blood Creatinine 72 hours						.745*						
Blood Glucose on Admission							0.025					
Blood Glucose 48 hours								0.109				
Blood Glucose 72 hours									0.196			
Blood Calcium on Admission										- 0,058		
Calcium Blood 48 hours											0.013	
Calcium Blood 72 hours												-,303*

* $P < 0.05$ (2 extremities). ICUs of HUC Curitiba, Paraná.

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sedation and MV, absence of calorie intake necessary for the maintenance of life, great volume losses, hemodynamic instability, and risk of infections [40–43].

Urea and creatinine are two substances present in blood and saliva which, when dosed, make evaluation of renal function possible [43,45]. In this study, elevated serum urea levels above the 72-hour normality parameters in Class II subjects and a decrease in creatinine at admission in Class I and at 48 and 72 hours in subjects in both classes were observed.

In view of the findings, it is pointed out that for proper renal functioning, it is necessary to evaluate four physiological functions: blood flow, glomerular filtration, tubular function, and permeability of the urinary tracts. One or more of the factors mentioned above are altered in polytraumatized individuals. The inadequate functioning of the kidneys predisposes an inability for glomerular filtration, causing an increase in urea and creatinine concentrations as a consequence of the increased protein catabolism present in polytraumatized individuals.

However, when serum levels, such as creatinine, are decreased, this also indicates severe changes in renal perfusion, which are usually caused by a decrease in renal blood flow and/or severe dehydration, excessive fluid replacement [40,41,46], situations experienced by individuals in both classes in this study.

Polytrauma patients have a rate ranging from 2% to 5% of risk of developing alterations in renal function, regardless of underlying trauma, due to the great influence of factors such as: hypovolemia, septic shock, aminoglycoside use, and contrast imaging tests, which usually progress to pre-renal and renal disorders [43,44,47,48]. Among the pre-renal causes, uremia related to an increase in protein catabolism and stress due to trauma, such as that observed in this study in relation to urea values 72 hours after admission in Class II individuals, stands out. Among the renal causes, acute tubular necrosis, usually caused by renal hypoperfusion and/or endogenous and exogenous nephrotoxins, are the most common factors during the care of critically ill ICU patients [40,42,43,44,46]. Information on renal function in these individuals in the ICU, through the control of electrolytes such as urea and creatinine, may contribute to the verification of situations that would lead to multiple organ failure during the hospitalization period, culminating in evolution and negative outcomes in relation to the prognosis [40,41,49]. Polytraumatized individuals of both classes I and II presented elevated levels of urea and salivary creatinine when compared to normality. Due to the lack of studies that perform this evaluation in polytrauma patients, normality for these two markers was drawn from values presented in previous studies performed with patients with chronic renal disease (CKD) on hemodialysis treatment and a control group that did not present with CKD [17–19]. Urea and creatinine are two substances present in the salivary flow and in the bloodstream that are dosed when there is a need to evaluate renal function in specific population groups (CKD and DM) [17,18,39]. Failure of renal function can occur due to the quality and intensity of aggressive stimuli to the kidneys, which causes loss of the functional unit of this organ, a scenario that is quite possible in polytraumatized patients in the ICU [39,40]. Salivary fluid has potential in other groups, since it is considered an excellent material for systemic verification and oral disease, among others [17,18,19,50].

The polytraumatized patients in this sample do not have CRF but may develop acute renal failure (ARF), which is a syndrome characterized by abrupt and persistent deterioration of renal function, resulting in the inability of the kidneys to excrete nitrogenous slags and to maintain hydroelectrolytic homeostasis [39]. Associated with this are the interventions used for resuscitation, such as the use of drugs that alter or make difficult glomerular filtration; and thus, the release of toxins in the body is a consequence [25,27, 51].

In view of the above, we stress that in our study salivary creatinine showed a very strong correlation in relation to the blood when dosed at admission; and a strong correlation in 48 h and 72 h, thus being a marker as effective as blood in acute situations and also having the advantage of early detection of changes.

Comparing the salivary urea of nephropathic individuals with normal individuals, it was identified in the first hour of fasting that the two samples presented a very high value when compared to blood urea [52]. Bearing in mind the investigation and the need for greater scientific confirmation regarding a special group of individuals such as those in this study, our findings are consistent with previous reports, even though in other categories we infer that it is important to highlight the possibility of using salivary creatinine and urea analysis for diagnosis and evaluation, not only of chronic kidney disease but also of acute renal disease, especially in polytrauma patients [18,19,52,53].

Serum glucose presented significant changes at the three times and in the two APACHE II classes, with values increasing in relation to the gold standard. Hyperglycemia is common in critical individuals and is attributed to the physiological response to trauma, due to the high degree of stress, represented by increased cortisol [54]. Glycemic levels are maintained physiologically by the interaction between insulin secretion, cellular uptake of glucose (glycolysis and glucogeneogenesis), hepatic glucose production (glycogenolysis and gluconeogenesis), and intestinal absorption [55–57]. Increased glycemia corresponds to increased metabolic demands in these individuals, and in most cases is accompanied by hyperinsulinemia and endogenous increase of liver production and also by causes such as increased glycemia in enteral and parenteral diets, dialytic solutions, and glucocorticoid use; vasopressor substances are routinely used in this study population [58–62].

According to the *American Disease Association* (ADA) and *Brazilian Society of Diabetes* (BDS), the reference value of fasting serum blood glucose obtained by puncture is less than 100 mg/dL for diabetic patients, and 100 to 125 mg/dL for diabetic patients with readings greater than or equal to 126 mg/dL (*National Diabetes Data Group*) [62–64]. Another common practice in the glycemic evaluation of patients in the ICU is a capillary check at the bedside, which can present the following results: lower than 70 mg/dL: hypoglycemia; 70 mg/dL to 140 mg/dL: normal; 140 mg/dL to 200 mg/dL: pre-diabetes; greater than 200 mg/dL: diabetes. These values have relevance, as long as they are obtained in the proper way with regard to the calibration technique [62–67]. The values considered here were 70 to 115 mg/dL [62]; the values of salivary glucose considered normal were: 5.6 to 18.4 mg/dL [68].

The glucose in the saliva presented an increase in the three times and in the two classes, with respect to the standard of normality. Studies indicate that saliva in type 2 diabetic individuals has potential for the detection of oral diseases as well as in the control of type 2 diabetes in follow-up [53]. Along these lines, both blood glucose and salivary glucose values converged, showing an increase in both. However, in the present study we did not verify correlation between serum and salivary glucose. A previous study [44] showed that in diabetic patients, the concentration of salivary glucose was much higher than in the control group.

Serum glycemic monitoring and its effects have been a concern in studies in the last decade. The verification of blood glucose and the mortality rates of critically ill patients in the ICU appear to have disparities in relation to three aspects: insulin administration, frequency of sample collection, and target amplitude of glycemic indexes [62,64,65]. Serum corrections for hyperglycemia were performed through blood and capillary serum evaluation. This fact may have interfered in the findings of this study, implying that these individuals could present a higher value of serum glucose in relation to what was found. Although this is one of the limitations of this study, it is emphasized that this intervention was performed in all individuals [66–68].

The serum calcium evaluated in this research is part of the group of essential mineral elements and needs to be acquired, mainly through food. The values of salivary calcium discussed here refer to the following readings: 5 to 7 mg/dL; however, these values are for healthy individuals, with the collection taken at rest–and they vary widely among individuals. As previously mentioned, this was the first study to evaluate the salivary index of polytraumatized patients in intensive care units (ICU), so there are no reference values to be compared [69].

The highest concentration of calcium in the human body occurs in the bone matrix (99%), distributed between bone and teeth in the form of calcium phosphate. The remaining calcium portion (1%) is located in the intra- and extracellular medium, mainly associated with the protein carrier, such as albumin. In polytraumatized individuals, bone lesions usually culminate in fractures and blood loss, compromising the production and balance of this element, which is vital to the proper functioning of the organism. There are several important functions attributed to calcium, mainly in the regulation of organic processes such as neuromuscular excitability, secretory processes, release of hormones, and neurotransmitters, besides the maintenance and formation of the bone matrix. In these situations, it acts as a transmitter of signs or as a protein activator. Maintaining calcium homeostasis is essential to the correct functioning of the body and also contributes to the better functioning of the other physiological systems [40,41,43].

In our study, calcium in Class I decreased at admission, increased at 48 hours, and decreased at 72 hours. Regarding Class II, calcium decreased at admission, increased at 48 hours, and normalized at 72 hours. Salivary calcium increased in relation to the normality pattern and, in relation to the times, it increased at 48 hours in Class II individuals compared to Class I. Due to their varied traumas and complex treatment, the individuals participating in this study may develop diseases that result in hypocalcemia or hypercalcemia. The calcium values presented here are in accordance with the literature. A decrease in calcium concentration after a trauma situation is associated with the fact that this individual will present hemodynamic changes, due to large volume losses, musculoskeletal trauma followed by fractures and stress, as well as vigorous replacements culminating with imbalances of the alkalosis type. In a study performed with trauma victims who presented a volume loss, serum calcium values behaved in a similar manner to that found, even when receiving adequate resuscitation when hospitalized (decrease, increase, stabilization with normality) [69].

In relation to the values found for salivary calcium, certain hypotheses may be proposed in order to explain this marked increase. The first is the high degree of stress present in the patients participating in the study, since they had suffered polytrauma. This higher salivary level of cortisol was verified in a previous study in adults in ICU with cranial trauma [23]. The autonomic nervous system regulates the salivation process, including the flow and concentration of certain salivary components, which provides a reliable measure of the sympathetic response [70]. Salivary flow can be altered by olfactory stimuli, exposure to luminosity, body position, and circadian cycle [14,15]. Another factor in the high level of calcium to be explained is that the intracellular calcium vesicles that act as second messenger, regulating various functions and being released on a strong hormonal stimulus, would be released in the salivary secretion because the patients are under a strong stimulus stressor [71]. Hormonal changes can have profound effects on metabolic homeostasis and circulatory hemodynamics and in the mechanisms of renal homeostasis and gastrointestinal physiology [72].

In addition to all the aforementioned functions, calcium, along with other minerals, promotes the maintenance of the isoelectric point of proteins, bone mineralization, transmission of nerve impulses, maintenance of the contraction mechanism and relaxation of the muscles, and also regulation of the ionic balance effect in enamel remineralization [52,65], thus guaranteeing temporary oral health in the patient. Because it is a transient state of high calcium, it also increases individuals' susceptibility to dental calculus formation.

Conclusion

This study provides evidence that salivary components can be used to follow clinical evolution in polytrauma patients admitted to the ICU.

Supporting information

S1 Text. Authorization of the Cajuru University Hospital.
(PDF)

S2 Text. Authorization for ICU study.
(PDF)

S3 Text. Consubstantiated opinion of CEP.
(PDF)

S4 Text. Free and informed consent form.
(PDF)

S5 Text. APACHE II Instrument.
(PDF)

S6 Text. Predictive mortality.
(PDF)

S7 Text. Coefficient of lethality.
(PDF)

S8 Text. Predictive value of APACHE II (APACHE II groups).
(PDF)

S9 Text. Data collection instrument.
(PDF)

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References

1. World Health Organization, Pan American Health Organization Brazil [World Health Organization, Pan American Health Organization Brazil]. Traumas kill more than the three major endemics: malaria, tuberculosis and AIDS. Brasília (DF): Pan American Health Organization Brazil; 2016 [cited 2018 Nov 15]. Available from: http://www.paho.org/bra. . ./index.php?option=com_content&view=article&id=2989. Portuguese.
2. World Health Organization. Injuries and violence: the facts [Internet]. Geneva: World Health Organization; c2010 [cited 2007 Mar 26]. 19 p. Available from: https://www.who.int/violence_injury_prevention/key_facts/en/.
3. Bacchieri G, Barros AJD. [Traffic accidents in Brazil from 1998 to 2010: many changes and few effects]. Rev Saude Publica [Internet]. 2011 Oct [cited 2018 Aug 12]; 45(5):949–63. Available from: http://www.scielo.br/scielo.php?pid=S0034-89102011000500017&script=sci_abstract&tlng=pt. Portuguese. https://doi.org/10.1590/s0034-89102011005000069 PMID: 21953026
4. Sociedade Brasileira de Atendimento Integral ao Traumatizado. Basta! Arguably, it can not be left alone in the speech. Trauma BoleTeam [Internet]. 2014 Jun 24 [cited 2018 Jun 15];Prevenção:[about 1 p.]. Available from: http://www.sbait.org.br/trauma.php. Portuguese.
5. American College of Surgeons, Advanced Trauma Life Support. About Advanced Trauma Life Support [Internet]. Chicago: American College of Surgeons; c2018 [cited 2018 Nov 5]. Available from: https://www.facs.org/quality-programs/trauma/atls/about.
6. Wilson JL, Herbella FAM, Takassi GF, Moreno DG, Tineli AC. Fatal trauma injuries in a brazilian big metropolis: a study of autopsies. Rev Col Bras Cir [Internet]. 2011 [cited 2018 Apr 18]; 38(2):122–6. Available from: http://www.scielo.br/scielo.php?pid=S0100-69912011000200010&script=sci_abstract&tlng=es. https://doi.org/10.1590/S0100-69912011000200010 PMID: 21710051
7. Krokoscz DVC. Effects of staff allocation and nursing workload on outcomes of care in medical-surgical units [8 thesis on the Internet]. São Paulo (SP): Universidade de São Paulo; 2007 [cited 2017 Nov 3].

101 p. Available from: http://www.teses.usp.br/teses/disponiveis/7/7139/tde-20062007-102806/pt-br.php. Portuguese.

8. Car MR. Nursing problems of the physical sphere in hospitalized patients: characterization by hospitalization units, semi intensive care and intensive treatment [thesis]. São Paulo (SP): School of Nursing, University of São Paulo; 1986. Portuguese.
9. Tranquitelli AM, Ciampone MHT. [Number of nursing care hours in an intensive care unit]. Rev Esc Enferm USP [Internet]. 2007 Sep [cited 2018 Jan 18]; 41(3):371–7. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0080-62342007000300005. Portuguese. https://doi.org/10.1590/s0080-62342007000300005 PMID: 17977372
10. Couto RC, Botoni FA, Serufo JC, Nogueira JM, Correa MM, Reis MAS, et al. Ratton-Emergências Médicas e Terapia Intensiva. Rio de Janeiro: Guanabara Koogan; 2005. Portuguese.
11. Cardozo Júnior LCM, Silva RR. Sepsis in intensive care unit patients with traumatic brain injury: factors associated with higher mortality. Rev Bras Ter Intensiva [Internet]. 2014 [cited 2017 Apr 20]; 26(2):148–54. Available from: http://www.scielo.br/scielo.php?pid=S0103-507X2014000200148&script=sci_abstract. https://doi.org/10.5935/0103-507X.20140022 PMID: 25028949
12. Santos AACS, Godoy MF. Critical analysis of the request for complementary exams in pediatrics. Pediatrics. 1999; 2(3):215–21. Portuguese.
13. Evia JRB. Inappropriate use of the clinical laboratory. Rev Mex Patol Clin [Internet]. 2003 Oct-Dec [cited 2017 Jan 29]; 50(4):209–23. Available from: https://www.medigraphic.com/pdfs/patol/pt-2003/pt034e.pdf. Spanish.
14. Mehari SM, Havill JH, Montgomery C. A written guideline implementation can lad to reductions in the laboratory testing in an intensive care unit. Anaesth Intensive Care [Internet]. 1997 Feb [cited 2018 Apr 11]; 25(1):33–7. Available from: https://aaic.net.au/document/?D=1996101. https://doi.org/10.1177/0310057X9702500106 PMID: 9075511
15. Zimmerman JE, Seneff MG, Sun X, Wagner DP, Knaus WA. Evaluating laboratory usage in the intensive care unit: patient and institutional characteristics that influence frequency of blood sampling. Crit Care Med [Internet]. 1997 May [cited 2018 Jun 22]; 25(5):737–48. Available from: https://insights.ovid.com/pubmed?pmid=9187590. https://doi.org/10.1097/00003246-199705000-00006 PMID: 9187590
16. Flabouris A, Bishop G, Williams L, Cunningham M. Routine blood test ordering for patients in the intensive care. Anaesth Intensive Care [Internet]. 2000 Oct [cited 2018 Oct 22]; 28(5):562–65. Available from: https://aaic.net.au/document/?D=2000031. https://doi.org/10.1177/0310057X0002800515 PMID: 11094676
17. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Crit Rev Oral Biol Med [Internet]. 2002 [cited 2018 Dec 11]; 13(2):197–212. Available from: https://journals.sagepub.com/doi/full/10.1177/154411130201300209. https://doi.org/10.1177/154411130201300209 PMID: 12097361
18. Dawes C. Considerations in the development of diagnostic tests on saliva. Ann N Y Acad Sci [Internet]. 1993 Sep [cited 1993 Dec 12]; 20(694):265–9. Available from: https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1993.tb18359.x?sid=nlm%3Apubmed https://doi.org/10.1111/j.1749-6632.1993.tb18359.x
19. Moura SAB. Clinical, sialometric and sialochemical analyzes in individuals with oral burning syndrome [dissertation]. João Pessoa (PB): Universidade Federal da Paraíba, Universidade Federal da Bahia; 2004. Portuguese.
20. Wang B, Du J, Zhu Z, Ma Z, Wang S, Shan Z. Evaluation of Parotid Salivary Glucose Level for Clinical Diagnosis and Monitoring Type 2 Diabetes 18 Mellitus Patients. Biomed Res Int [Internet]. 2017 Jan 5 [cited 2018 Jan 22]; 2017(1):1–5. Available from: https://www.hindawi.com/journals/bmri/2017/2569707/. https://doi.org/10.1155/2017/2569707
21. Kovalč´ıkova A, Jansákova K, Gyurászová M, Podracká L, Sebeková K, Celec P, et al. Salivary creatinine and urea higher in an experimental model of acute but not chronic renal disease. PLoS One [Internet]. 2018 Jul 6 [cited 2018 Jan 12]; 13(7):1–11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6034877/pdf/pone.0200391.pdf. https://doi.org/10.1371/journal.pone.0200391 PMID: 29979784
22. Tzira D, Prezerakou A, Papadatos I, Vintila A, Bartzeliotou A, Apostolakou F, et al. Salivary Biomanrkers May Mensure Stress Responses in Critically ill Children. SAGE Open Med [Internet]. 2018 Sep 25 [cited 2018 Jul 16]; 6:1–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6156207/. https://doi.org/10.1177/2050312118802452 PMID: 30263122
23. Bartanusz V, Corneille MG, Sordo S, Gildea M, Michalek JE, Prakash VN, et al. Diurnal salivar cortisol measurement in the neurosurgical-surgical 30 intensive care unit in critically ill acute trauma patients. J Clin Neurosci [Internet]. 2014 Dec [cited 2017 Oct 22]; 21(12):2150–4. Available from: https://doi.org/10.1016/j.jocn.2014.04.018 PMID: 25065844

24. Rodrigues PV, Franco MM, Marques CPC, Carvalho RCC, Leite SAM, Pereira ALA, et al. Salivary levels of calcium, phosphorus, potassium, albumin and correlation with serum biomarkers in hemodialysis patients. Arch Oral Biol [Internet]. 2016 Feb [cited 2018 Apr 24]; 62:58–63. Available from: https://www.sciencedirect.com/science/article/pii/S0003996915300881?via%3Dihub. https://doi.org/10.1016/j.archoralbio.2015.11.016 PMID: 26655748

25. Knaus WA, Zimmermann JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. Crit Care Med. 1981 Aug; 9 (8):591–7. https://doi.org/10.1097/00003246-198108000-00008 PMID: 7261642

26. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985 Oct; 13(10):818–17. PMID: 3928249

27. Knaus WA, Wagner DP, Draper EA, Zimmerman JE, Bergner M, Bastos PG, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest [Internet]. 1991 Dec [cited 2018 May 15]; 100(6):1619–36. Available from: https://doi.org/10.1378/chest.100.6.1619 PMID: 1959406

28. Resolution RDC No. 7 of February 24, 2010 (BR). It provides for the minimum requirements for the operation of Intensive Care Units and provides other measures. 2010 Feb 25 [cited 2018 Jan 18]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2010/res0007_24_02_2010.html. Portuguese.

29. Portaria nº 3.432 of 12, of August of 1998 (BR). Establishes classification criteria for Intensive Care Units (ICUs). 1998 Aug 13 [cited 2018 Jun 1]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/gm/1998/prt3432_12_08_1998.html. Portuguese.

30. Ordoñez CA, Badiel M, Sánchez AI, Granados M, García AF, Ospina G, et al. Improving mortality predictions in trauma patients undergoing damage control strategies. Am Surg [Internet]. 2011 Jun [cited 2018 Apr 9]; 77(6):778–82. Available from: https://www.ingentaconnect.com/content/sesc/tas/2011/00000077/00000006/art00037%3bjsessionid=15ow9f51g9rr0.x-ic-live-02. PMID: 21679650

31. Zimmerman JE, Kramer AA, McNair DS, Malila FM. Acute Physiology and Chronic Health Evaluation (APACHE) IV: hospital mortality assessment for today's critically ill patients. Crit Care Med [Internet]. 2006 May [cited 2018 Nov 5]; 34(5):1297–310. Available from: https://insights.ovid.com/pubmed?pmid=16540951. https://doi.org/10.1097/01.CCM.0000215112.84523.F0 PMID: 16540951

32. Canova JCM, Bueno MFR, Oliver CCD, Souza LA, Belati LA, Cesarino CB, et al. Cranioencephalic trauma of patients who have been victims of motorcycle accidents. Arq Ciênc Saúde [Internet]. 2010 Jan-Mar [cited 2017 Oct 1]; 17(1):9–14. Available from: http://repositorio-racs.famerp.br/racs_ol/vol-17-1/IDL_jan-mar_2010.pdf. Portuguese.

33. Chalya PL, Gilyoma JM, Dass RM, Mchembe MD, Matasha M, Mabula JB, et al. Trauma admissions to the intensive care unit at a reference hospital in Northwestern Tanzania. Scand J Trauma Resusc Emerg Med [Internet]. 2011 Oct [cited 2018 Feb 20]; 19:61. Available from: https://doi.org/10.1186/1757-7241-19-61 PMID: 22024353

34. Gross T, Attenberger C, Huegli RW, Amsler F. Factors associated with reduced longer-term capacity to work in patients after polytrauma: a Swiss trauma center experience. J Am Coll Surg [Internet]. 2010 Jul [cited 2018 Feb 3]; 211(1):81–91. Available from: https://doi.org/10.1016/j.jamcollsurg.2010.02.042 PMID: 20610253

35. Tolotti VC, Silva LAA. Characterization of the victims of trauma treated in a hospital emergency in the north of the state of Rio Grande do Sul. Rev Contexto & Saúde [Internet]. 2004 Jul-Dec [cited 2018 Dec 2]; 4(7):191–8. Available from: https://www.revistas.unijui.edu.br/index.php/contextoesaude/article/view/1338. https://doi.org/10.21527/2176-71142004.07.191–198

36. Costa JI, Amaral JLG, Munechika M, Juliano Y, Bezerra Filho JG. Severity and prognosis in intensive care: prospective application of the APACHE II index. Sao Paulo Med J [Internet]. 1999 Sep [cited 2018 Jan 28]; 117(5):205–14. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-31801999000500005. https://doi.org/10.1590/s1516-31801999000500005 PMID: 10592133

37. Bales ME, Johnson SB, Keeling JW, Carley KM, Kunkel F, Merrill JA. Evolution of coauthorship in public health services and systems research. Am J Prev Med [Internet]. 2011 Jul [cited 2017 Sep 20]; 41 (1):112–7. Available from: https://doi.org/10.1016/j.amepre.2011.03.018 PMID: 21665073

38. Santos AMR, Moura MEB, Nunes BMVT, Leal CFS, Teles JBM. [Profile of motorcycle accident victims treated at a public hospital emergency department]. Cad Saude Publica [Internet]. 2008 Aug [cited 2018 Jul 10]; 24(8):1927–38. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-311X2008000800021. https://doi.org/10.1590/s0102-311x2008000800021 Portuguese. PMID: 18709233

39. Guyton AC, Hall JE. Treaty of Medical Physiology. Rio de Janeiro: Elsevier; 2006. Portuguese.

40. Kellum JA. Disorders of acid-base balance. Crit Care Med [Internet]. 2007 Nov 1 [cited 2018 Dec 1]; 35 (11):2630–6. Available from: https://europepmc.org/abstract/med/17893626. https://doi.org/10.1097/01.CCM.0000286399.21008.64 PMID: 17893626

41. Évora PRB, Garcia LV. [Acid-base balance]. Medicina [Internet]. 2008 [cited 2017 Oct 20]; 41(3):301–11. Available from: http://www.revistas.usp.br/rmrp/article/view/275/276. Portuguese.
42. Association of Brazilian Intensive Medicine, Mechanical Ventilation Committee; Brazilian Society of Pulmonology and Tisiology, SBPT Intensive Care Committee. Brazilian Guidelines on Mechanical Ventilation-2013 [Internet]. [São Paulo]: Association of Brazilian Intensive Medicine; 2013 [cited 2018 Oct 27]. 136 p. Available from: https://edisciplinas.usp.br/pluginfile.php/237544/mod_resource/content/1/Consenso%20VM%202013.pdf.
43. Riella MC. Principles of nephrology and hydroelectrolytic disorders. 4. ed. Rio de Janeiro: Guanabara Koogan; 2003. Portuguese.
44. Palevsky PM, Metnitz PG, Piccinni P, Vinsonneau C. Selection of endpoints 13 for clinical trials of acute renal failure in critically ill patients. Curr Opin Crit Care [Internet]. 2002 Dec [cited 2018 Jul 2]; 8(6):515–8. Available from: https://insights.ovid.com/pubmed?pmid=12454535. PMID: 12454535
45. Greabu MI, Battino M, Mohora M, Totan A, Didilescu A, Spinu T, et al. Saliva-a diagnostic window to the body, both in health and in disease. J Med Life [Internet]. 2009 Apr-Jun [cited 2018 Dec 3]; 2(2):124–32. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3018981/. PMID: 20108531
46. Lima OPSC. Reading and interpretation of nursing exams. 3. ed. Goiânia: AB; 2008. Portuguese.
47. Garcia TPR, Romero MP, Poletti NAA, Cesarino CB, Ribeiro RCHM. [Main Reasons of Patient's Hospitalization with Acute Renal Failure in Intensive Care Unit]. Arq Clinc Saúde [Internet]. 2005 Jul-Sep [cited 2018 Jun 11]; 12(3):146–50. Available from: http://repositorio-racs.famerp.br/racs_ol/vol-12-3/05%20-%20ID108.pdf. Portuguese.
48. Batista PBP, Santos OFP. IRA Prognoses. In: Schor N, Boim MA, Santos OFP. Acute renal failure. 2. ed. São Paulo: Sarvier; 1997. p. 333–51. Portuguese.
49. Gamarra G, Dí Achiardi RR, Ordonez JD, Torres IH. Acute renal failure. Acta Med Colomb. 1981; 6 (1):17–22.
50. Moura SAB, Medeiros AMC, Costa FRH, Moraes PH, Oliveira Filho SA. [Diagnostic Value of Saliva in Oral and Systemic Diseases: A Literature Review]. Pesqui Bras Odontopediatria Clin Integr [Internet]. 2007 May-Aug [cited 2018 May 2]; 7(2):187–94. Available from: http://revista.uepb.edu.br/index.php/pboci/article/view/200/147. https://doi.org/10.4034/pboci.v7i2.200 Portuguese.
51. Polita JR, Gomez J, Friedman G, Ribeiro SP. Comparison of APACHE II and 19 three abbreviated APACHE II scores for predicting outcome among emergency trauma patients. Rev Assoc Med Bras [Internet]. 2014 [cited 2017 Dec 1]; 60(4):381–386. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-42302014000400381. https://doi.org/10.1590/1806-9282.60.04.018 PMID: 25211423
52. Bilancio G, Cavallo P, Lombardi C, Guarino E, Cozza V, Giordano F, et al. Salivary levels of phosphorus and urea as indices of their plasma levels in nephropathic patients. J Clin Lab Anal [Internet]. 2018 Sep [cited 2018 Dec 10]; 32(7):224–49. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jcla.22449. PMID: 29603373
53. Mata AD, Marques D, Rocha S, Francisco H, Santos C, Mesquita MF, et al. Effects of diabetes mellitus on salivary secretion and its composition in the human. Mol Cell Biochem [Internet]. 2004 Jun [cited 2017 Apr 28]; 261(1):137–42. Available from: https://link.springer.com/article/10.1023/B:MCBI.0000028748.40917.6f.
54. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest [Internet]. 2004 Nov [cited 2017 Oct 12]; 114(9):1187–95. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC524243/. https://doi.org/10.1172/JCI23506 PMID: 15520847
55. Montori VM, Bistrian BR.; McMahon MM. Hyperglycemia in Acutely Ill Patients. JAMA [Internet]. 2002 Nov [cited 2018 Abr 23]; 288(17):2167–69. Available from: https://jamanetwork.com/journals/jama/article-abstract/195453. https://doi.org/10.1001/jama.288.17.2167 PMID: 12413377
56. Inzucchi SE, Siegel MD. Glucose Control in the ICU–How Tight Is Too Tight? N Engl J Med [Internet]. 2009 Mar [cited 2018 Abr 23]; 360:1346–9. Available from: https://www.nejm.org/doi/full/10.1056/NEJMe0901507. PMID: 19318385
57. Critchell CD, Savarese V, Callahan A, Aboud C, Jabbour S, Marik P. Accuracy of bedside capillary blood glucose measurements in critically Ill patients. Intensive Care Med [Internet]. 2007 Dec [cited 2018 May 4]; 33(12):2079–84. Available from: https://link.springer.com/article/10.1007%2Fs00134-007-0835-4. https://doi.org/10.1007/s00134-007-0835-4 PMID: 17763842
58. Cook A, Laughlin D, Moore M, North D, Wilkins K, Wong G, et al. Differences in Glucose Values Obtained from Point-of-Care Glucose Meters and Laboratory Analysis in Critically Ill Patients. Am J Crit Care [Internet]. 2009 Jan [cited 2018 May 4]; 18(1):65–72. Available from: http://ajcc.aacnjournals.org/content/18/1/65.short. https://doi.org/10.4037/ajcc2009626 PMID: 19116407
59. Australian and New Zealand Intensive Care Society Clinical Trials Group; George Institute for International Health; Canadian Critical Care Trials Group; Vancouver Coastal Health Research Institute.

Intensive versus Conventional Glucose Control in Critically Ill Patients. N Engl J Med [Internet]. 2009 Mar [cited 2018 Abr 23]; 360:1283–97. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa0810625. PMID: 19318384

60. Brunner R, Adelsmayr G, Herkner H, Madl C, Holzinger U. Glycemic variability and glucose complexity in critically ill patients: a retrospective analysis of continuous glucose monitoring data. Crit Care. [Internet]. 2012 Out [cited 2018 May 4]; 16(5):R175. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682275/. https://doi.org/10.1186/cc11657 PMID: 23031322

61. Inoue S, Egi M, Kotani J, Morita K. Accuracy of blood-glucose measurements using glucose meters and arterial blood gas analyzers in critically ill adult patients: systematic review. Crit Care [Internet]. 2013 Mar [cited 2018 May 4]; 17(2):R48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3672636/. https://doi.org/10.1186/cc12567 PMID: 23506841

62. Diabetes in Control. A Summary of ADA's New 2018 Standards of Medical Care in Diabetes [Internet]. [place unknown]: Diabetes in Control; 2017 Dec 16 [cited 2018 nov 12]. Available from: http://www.diabetesincontrol.com/a-summary-of-adas-new-2018-standards-of-medical-care-in-diabetes/.

63. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes–2018. Diabetes Care [Internet]. 2018 Jan; 41(Suppl 1):S13–S27 Available from: http://care.diabetesjournals.org/content/41/Supplement_1/S13.long. https://doi.org/10.2337/dc18-S002 PMID: 29222373

64. Krinsley JS. Effect of an Intensive Glucose Management Protocol on the Mortality of Critically Ill Adult Patients. Mayo Clin Proc [Internet]. 2004 Ago [cited 2018 Apr 23]; 79(8):992–1000. Available from: https://doi.org/10.4065/79.8.992 PMID: 15301325

65. Holzinger U, Warszawska J, Kitzberger R, Wewalka M, Miehsler W, Herkner H, et al. Real-Time continuous glucose monitoring in critically ill patients: a prospective randomized trial. Diabetes Care [Internet]. 2010 Mar [cited 2018 May 4]; 33(3):467–72. Available from: http://care.diabetesjournals.org/content/33/3/467.long. https://doi.org/10.2337/dc09-1352 PMID: 20007948

66. Wiener RS; Wiener DC, Larson RJ. Benefits and Risks of Tight Glucose Control in Critically Ill Adults: a Meta-analysis. JAMA [Internet]. 2008 Aug [cited 2018 Apr 23]; 300(8):933–44. Available from: https://jamanetwork.com/journals/jama/fullarticle/182432. https://doi.org/10.1001/jama.300.8.933 PMID: 18728267

67. Ichai C, Preiser JC; Société Française d'Anesthésie-Réanimation; Société de Réanimation de langue Française; Experts group. International recommendations for glucose control in adult non diabetic critically ill patients. Crit Care [Internet]. 2010 Set [cited 2018 May 10]; 14(5):R166. Available from: https://ccforum.biomedcentral.com/articles/10.1186/cc9258. PMID: 20840773

68. Darwazeh AM, MacFarlane TW, McCuish A, Lamey PJ. Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. J Oral Pathol Med [Internet]. 1991 Jul [cited 2018 May 2]; 20(6):280–3. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-0714.1991.tb00928.x?sid=nlm%3Apubmed. PMID: 1890663

69. Webster S, Todd S, Redhead J, Wright C. Lonised calcium levels in major trauma patients who received blood in the Emergency Department. Emerg Med J [Internet]. 2016 Aug [cited 2018 Sep 6]; 33(8):569–72. Available from: https://emj.bmj.com/content/33/8/569.long. https://doi.org/10.1136/emermed-2015-205096 PMID: 26848163

70. Chiappelli F, Iribarren FJ, Prolo P. Salivary biomarkers in psychobiological medicine. Bioinformation [Internet]. 2006 Dec 29 [cited 2018 Oct 20]; 1(8):331–4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1891707/. https://doi.org/10.6026/97320630001331 PMID: 17597915

71. Lima DP, Correia ASC, Anjos AL, Boer NP. [Use of saliva for diagnosis of oral and systemic diseases]. Rev Odontol Arac [Internet]. 2014 Jan-Jun [cited 2018 Dec 1]; 35(1):55–9. Available from: http://apcdaracatuba.com.br/revista/2014/10/v35n122014.htm. Portuguese.

72. Melo REVAM, Vitor CMA, Silva MBL, Luna LAL, Firmo ACB, Melo MMVA. [Hormonal answer in the polytraumatized patient]. Int J Dent [Internet]. 2005 Jan-Jun [cited 2018 Jul 22]; 4(1):31–6. Available from: https://periodicos.ufpe.br/revistas/dentistry/article/view/13844/16693. Portuguese.