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Dysregulation of Cell-Mediated Immunity in Patients with Long ICU Stay Affected of Multiple Organ Dysfunction Syndrome and Multifocal Candidiasis

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Abstract

Introduction: The endogenous immunosuppression in critically ill patients with Multiple Organ Dysfunction Syndrome, requiring long-term ICU, can play an important role in the prognosis of Multifocal Candidiasis.

Method: Prospective study with control group (33 healthy volunteers) and study group (43 critically ill patients non neutropenic admitted to ICU with invasive mechanical ventilation), about cellular immunity *in vitro*, using Lymphoblastic Transformation Tests (LTT) stimulated with phytohemagglutinin (PHA). Simultaneously, the study group is followed up on appearance of Invasive Candidiasis and *in vivo* immunity study, using skin tests during the first week of ICU stay.

Results: There are significant statistical differences between the control group and the study group in relation to LTT PHA results. These differences are also found in the study group in relation to mortality and when skin tests are negative. When analysing the subgroup of patients with ICU stay longer than 12 days, there are statistically significant differences in LTT PHA when comparing the test performed in the 1st week in ICU in survivor patients with Invasive Candidiasis and the control group, but there are no differences with the 2nd determination. There are statistically significant differences when comparing the results of LTT PHA among patients with Invasive Candidiasis according to mortality in the 2nd determination, but these differences were not identified in the 1st week of ICU.

Conclusions: Both *in vivo* cellular immunity studies, using skin tests, and *in vitro* studies with the LTT PHA, help to establish a prognosis in critically ill patients with Multiple Organ Dysfunction Syndrome requiring long-term ICU care and in which a Multifocal Candidiasis has been identified. Monitoring of T-cell dysregulation and endogenous immunosuppression can help to identify patients who may benefit from new immunomodulatory therapeutic strategies.

Keywords: *Cell-immunity, Multifocal candidiasis, Multiple organ dysfunction syndrome, Non-neutropenic, ICU*

Introduction

In order to make Multifocal Candidiasis equivalent to Invasive Candidiasis, and thus initiate an early antifungal treatment to prevent death attributable to *Candida spp*. in non-neutropenic in critical ICU patients who are neither neutropenic nor affected by AIDS [1,2], we analysed the factors that could contribute to the transformation from colonization of *Candida spp.* [2] to Multifocal Candidiasis and subsequent disseminated candidiasis. It is universally accepted that *Candida spp.* infections are linked to the antibiotic era [3]. The prolonged use of broad-spectrum antibiotics leads to an intestinal microbiological imbalance, promoting an excessive growth of candida in the intestinal lumen and thus facilitating the translocation of these fungi into the interior of the organism [4]. As an example, in the observations that were made, it was very common that, after treating with antibiotics treatment with broad-spectrum antibiotics for a sepsis due to *Pseudomonas spp*., if the patient survived and remained in critical condition, it was very common to detect the appearance of an invasive candidiasis. However, in these ICU patients who remain in a critical condition for a prolonged period of time (more than 7 days), other factors related to ICU treatment and monitoring can be identified, which should be considered as facilitators of Multifocal Candidiasis [5]:

- Gastrointestinal dysfunction: Malnutrition and/or absence of enteral nutrition due to intestinal paresis; breakdown of the intestinal physiological barrier; treatment with parenteral nutrition, gastric protectors and prolonged nasogastric tube
- Prolonged vesical catheterization
- Prolonged arterial or venous catheter
- Mechanical ventilation more than 7 days
- Hemofiltration techniques

In some cases, this list can also be added treatment with prolonged treatment or high doses of corticosteroids (30% in these patients) [6]. It is at this point where it is questioned whether there may be in these patients an immunodeficiency acquired in the ICU and that this may play a role as important as the prolonged antibiotic treatment.

Methods and Materials

Prospective Study with Control Group

Study Group (43 patients): Patients over 18 years old and middle ages of 61 ± 10,8 (37-86), admitted to ICU with invasive mechanical ventilation without history of neutropenia or AIDS, and need for mechanical ventilation for more than 48h. There was no informed consent from the patients because their level of consciousness on admission to the ICU does not allow it. Family members have been informed about participation in the study, its risks and that this participation will not modify any of the protocols required in patient care throughout their stay in the ICU. Only the patients with the consent of their relatives have been included. Mortality rate of this study population was 46%. In 12 cases the UCI stay was lesser than 12 days with 42% mortality rate, and in 31 cases UCI stay was more than 12 days with a 48% mortality rate. The diseases that were the reason for ICU admission, grouped as follows were:

- Chronic Obstructive Pulmonary Disease with respiratory failure requiring invasive mechanical ventilation prior to ICU admission (15 cases)
- Postoperatory complicated cardiac or abdominal surgery with more than 24 h. of mechanical ventilation (12 cases)
- Patients with Tetanus disease with invasive mechanical ventilation (13 cases).

Control Group (33 healthy volunteers): Group formed by subjects who have been informed prior to the acceptance of being included in this study.

Immunity Cellular Evaluation

1. *In vivo* immunity study using skin tests, only in the study group. The test is considered positive if they present, at least in one of the puncture sites, an induration greater than 5 mm in diameter against the selected antigens (Streptokinase-Streptodornase 1/4, Candidin 1/500, P.P.D. 1/1000, Toxoplasmin). The test is performed during the first week of ICU stay and is not repeated at any other time.
2. *In vitro* immunity study using Lymphoblastic Transformation Tests (LTT), lymphocyte extraction is performed in both groups. Technique: An adjustment of lymphocytes to 2x106 cells/ml is performed. Three control cultures and three stimulated with phytohemagglutinin (PHA) are grown for 72 h. at 37°C, with aerobic culture and 5% CO2. After this time, the slide extension and May-Grünwald, Giemsa staining is performed. Once the staining is done, the percentage of blasts over the total population of lymphocytes is calculated. This test, in the study group, is performed during the first 6 days of stay in the ICU and then every 12-17 days until discharge from the ICU.

Diagnosis of Multifocal Candidiasis

From the inclusion of the patients in the study group, cultures to identify *Candida spp*. are carried out once a week, until the ICU is discharged, to identify *Candida spp.* in five *foci*:

1. Blood cultures
2. Respiratory secretions from tracheobronquial aspiration
3. Urinary cultures from closed circuit bladder catheterization. Positive only with more than 5000 colonies/ml.
4. Digestive. Simultaneous presence of positive cultures in the pharyngeal swab and in the gastric aspirate obtained through the nasogastric tube
5. Other *foci*. Positive cultures from drainages or wound exudates

Candida colonization is defined when this study demonstrates the presence of *Candida spp*. in only one of these *foci* and the blood culture is negative. Candida multifocality or Invasive Candidiasis is defined when positive cultures are identified simultaneously in two or more of these *foci* and/or positive blood cultures.

Variables and Statistical Analysis Methodology

The following variables were analysed: ICU stay, ICU outcome, presence or absence of *Candida spp.* multifocality, presence of positive skin tests and results of LTT stimulated with PHA. Data were analysed using the SPSS statistical program. Categorical variables were compared among groups with Chi-square or Fisher exact test as appropriate. Continuous variables were analysed with the Student's *t* or Mann-Whitney U test when the distributions departed from normality and described as mean or median (standard deviation or variance). Statistical significance was established at p value < 0.05 on two-tailed testing.

Results

No significant differences were detected between the number of days spent in the ICU and mortality (42% mortality in stays of less than 13 days vs. 48% in stays of more than 12 days, $X^2 = 0.16$). Significant differences were detected between patients according to whether or not they had positive skin tests and mortality (mortality of 27.8% in patients with positive skin tests vs. 78.3% with negative skin tests, $X^2 = 10.45$ ($p = 0.001$)) and between presence of multifocal candidiasis and mortality (30% mortality in patients with colonization vs. 73.9% in patients with multifocality, $X^2 = 8.29$ ($p = 0.004$)). These differences are more significant if only the group with stays longer than 12 days is analysed (10% mortality in patients with colonization vs. 71.4% in patients with multifocalities, $X^2 = 10.24$ ($p = 0.001$)). There were no significant differences between positive skin tests and the presence of Multifocal Candidiasis (50% of patients with colonization had negative skin tests vs. 60.9% of patients with multifocality, $X^2 = 0.48$).

Related on the LTT PHA done:

1. There are significant statistical differences between the control group and the study group, in relation to mortality (LTT PHA control group (group C) = 39.25% (6.79), LTT PHA study group survivors (group S) = 29.22% (11.67), LTT PHA

study group dead (group D) = 22.89% (13.11). Statistical differences between group C and group S: t = 3.86 (65 d.f.), p = 4x10^{-4}; between group C and group D: t = 6.29 (65 d.f.), p = 6x10^{-8}; between group S and group D: t = 2.45 (66 d.f.), p = 0.002). Statistically significant differences are not found when comparing the results of the LTT PHA of the first determination between group S and group D (LTT PHA group S = 27.29% (10.15), LTT PHA group D = 22.68% (12.33), t = 1.49 (34 d.f.), p = 0.131).

2. There were significant statistical differences between the control group and the study groups, whether the skin tests were positive or negative (LTT PHA control group (group C) = 39, 25% (6.79), LTT PHA study group with positive skin tests (group P) = 30.03% (12.16), LTT PHA study group with negative skin tests (group N) = 21.52% (12.41). Statistical differences between group C and group P: t = 3.8 (66 d.f.), p = 0.001; between group C and group N: t = 6.98 (60 d.f.), p = 5x10^{-9}; between group P and group N: t = 3.39 (62 d.f.), p = 0.002). There were significant statistical differences when comparing the results of LTT PHA of 2nd determination between group P and group N (LTT PHA group P = 30, 07% (10.73) and LTT PHA group N = 19,28% (10,59), t = 2,13 (18 d.f.), p = 0.025). Statistically significant differences are not found when comparing the results of the LTT PHA of the 1st determination between group P and group N (LTT PHA group P = 29.68% (8.27) and LTT PHA group N = 22.53% (13.05), t = 1.5 (34 d.f.), p = 0.129).

3. When analysing the subgroup of patients with ICU stay of more than 12 days, there were significant statistical differences between the control group and the study group according to survivors with colonization (1st study LTT PHA: t = 3,8 (39 d.f.), p = 0,001; 2nd study LTT PHA: t = 3,461 (35 d.f.), p = 0.002) and dead with multifocality for *Candida spp* (1st study LTT PHA: t = 5,82 (41 d.f.), p = 1,3x10^{-6}; 2nd study LTT PHA: t = 6,09 (43 d.f.), p = 4,5x10^{-7}). There were only significant statistical differences between the control group and the study group according to survivors with multifocality for *Candida spp* in the 1st study LTT PHA (1st study LTT PHA: t = 3,957 (37 d.f.), p = 0,0005; 2nd study LTT PHA: t = 0,603 (35 d.f.), p = 0,329). There were only significant statistical differences between dead with multifocality for *Candida spp.* and survivors with multifocality for *Candida spp.* in the 2nd study LTT PHA (1st study LTT PHA: t = 0,751 (14 d.f.), p = 0,291; 2nd study LTT PHA: t = 3,764 (14 d.f.), p = 0.002) (Table 1). There were no patients with colonization by *Candida spp.* who died more than 12 days.

Discussion

Critical illness is defined by presence of altered organ function in acutely ill patients such that homeostasis cannot be maintained without medical intervention in ICU, such as mechanical ventilation, vasoactive support for hemodynamic, renal replacement therapy, and so on [7]. The Sequential Organ Failure Assessment (SOFA) score is a simple method of assessing and monitoring organ dysfunction in critically ill patients [8,9]. The prognostic value of skin tests in critically ill patients has been observed for years [10]. There is also evidence of the prognostic value of Multifocal Candidiasis in these non-neutropenic critically ill patients. The detection of Multifocal or Invasive Candidiasis [2] can be an indicator of acquired immunosuppression in ICU patients affected by a Multiple Organ Dysfunction Syndrome [10]. In the patients of the study, the need for mechanical ventilation for more than 24 h. together with continuous sedation, before identifying Multifocal Candidiasis, indicates that their SOFA score is higher than 5. This fact confirms that the selected patients are admitted to the ICU with a Multiple Organ Dysfunction Syndrome. The LTT PHA carried out in this study demonstrate that the cellular immunity of non-neutropenic patients requiring mechanical ventilation for more than 48 h. presented significantly lower values than in a control group of non-neutropenic subjects. These results confirm the existence of an immunological response known as endogenous immunosuppression [11] in non-neutropenic critically ill patients. At the same time, these statistically significant differences disappear in the 2nd determination of the LTT PHA in critically ill patients with an ICU stay of more than 12 days and who survive. Therefore, improvement of this T-cell dysregulation [12] will condition ICU outcome in patients who remain in critical condition for more than 12 days. This observation raises the possibility that quantitative and qualitative kinetic monitoring of T-lymphocytes in ICU patients may help to identify those who may benefit from new immunomodulatory therapeutic strategies [13]. A possibility of this monitoring can be found following the proposal of the REALIST score [14].

Conclusions

Both *in vivo* cellular immunity studies, using skin tests, and *in vitro* studies with the LTT PHA help to establish a prognosis in critically ill patients requiring long-term ICU care and in which a Multifocal Candidiasis has been identified. Monitoring of T-cell dysregulation and endogenous immunosuppression can help to identify patients who may benefit from new immunomodulatory therapeutic strategies.

Table 1: LTT PHA results, 1st determination and 2nd determination, comparing control group with patients staying in the ICU for more than 12 days in the study group, collected according to mortality and presence or not of Multifocal Candidiasis.

	Characteristics of the groups	**LTT PHA 1st determination**			**LTT PHA 2nd determination**		
		Number of cases	**Median**	**Variance**	**Number of tests**	**Median**	**Variance**
Control Group	Healthy volunteers	33	39.25%	46	33	39.25%	46
Study Group with days of UCI stay > 12	Survivors and Candida spp colonization	8	27.69%	104.62	4	26.70%	30.99
	Survivors and multifocal candidiasis	6	25.75%	111.35	4	37%	54.67
	Dead and multifocal candidiasis	10	20.75%	166.17	12	20.78%	163.54

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