Psychological intervention based on psychoneuroimmunology improves clinical evolution, quality of life, and immunity of children with leukemia: A preliminary study

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Abstract

We conducted a non-randomized, open-label clinical trial to assess whether a psychoneuroimmunology-based intervention enhanced immunity in children with acute lymphoblastic leukemia undergoing chemotherapy. In total, 16 children (44% female) received psychoneuroimmunology-based intervention, whereas 12 (50% female) received health psychoeducation (controls). The primary outcome was immunity markers, being clinical conditions the secondary outcome. Psychoneuroimmunology-based intervention increased immune markers (CD8+ T, B, and natural killer cells, serum immunoglobulin A, and immunoglobulin M) and quality of life, whereas it shortens the duration of fever and use of antipyretics, antibiotics, analgesics, and respiratory therapy. Immunity markers correlated with clinical conditions. Thus, psychoneuroimmunology-based intervention could reduce hospital cost and increase patient well-being.

Introduction

Beliefs, attitudes, spirituality, and psychological perspectives can dramatically affect our health, disease course, and overall well-being. Psychological and social disorders are also capable of altering the immune system, which may influence vulnerability to the disease and its evolution (Danese and Lewis, 2017; Klinger et al., 2005; O'Connor et al., 2014). Different types of psychosocial interventions can improve quality of life (QoL), psychosocial adjustment, and clinical aspects of the disease, and possibly prolong survival time in cancer patients (Antoni, 2013; Fawzy et al., 1993; Kazak and Noll 2015; Simonton and Matthews-Simonton, 1981; Spiegel et al., 1989). Andersen et al. (2010) reported that psychosocial interventions could improve indicators of psychological adjustment (e.g. negative affect and social support), but in addition, these interventions can enhance immune function (e.g. lymphocyte proliferation) and reduce markers of inflammation in cancer patients. Interestingly, a better immunity has been associated with improved psychological aspects and habits of patients, but not with functional status, symptomatology, adverse effects of chemotherapy, and laboratory values (Andersen et al., 2004).Thus, it was proposed that changes in immunity induced by psychosocial interventions do not modify the severity of symptoms and the state of functionality; instead, those

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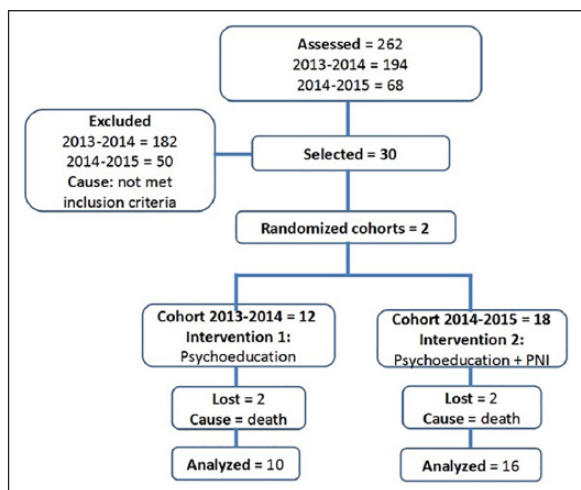Email: josymarchacin@yahoo.es

Figure 1. TREND (Transparent Reporting of Evaluations with Nonrandomized Designs) flowchart of the study.

changes induce behavioral modifications, especially reduction of distress (Andersen et al., 2007).

The psychosocial interventions of the studies reported so far have been basically designed to influence psychological aspects, social adaptation, and education about the disease, but they have not been developed to attempt to produce changes in specific components of the immune system. It is known that experimental psychological conditioning may influence immune system responses in both animals and healthy humans (Cohen et al., 1994; Giang et al., 1996; Green McDonald et al., 2013). Also, chemogenetic activation of the dopaminergic reward system (associated with positive emotions) lessens the sympathetic noradrenergic input to the bone marrow, which makes myeloid-derived suppressor cells less immunosuppressive and tumor-promoting, and as a result, tumor weight is reduced in animal models (Ben-Shaanan et al., 2018). This finding suggests that stimulation of positive emotions, in addition to a reduction in negative emotions, could be a desirable feature of any psychological intervention in cancer patients.

There are very few studies on psychiatric interventions based on psychoneuroimmunology-based intervention (PNI) reported in pediatric patients. In this study, we administered a psychological intervention, specifically directed toward cognitive processes related to immunity, to children with acute lymphocytic leukemia (ALL) during induction-to-remission chemotherapy, which is the first phase of treatment and is considered the most determinant period for the prognosis (Salgado et al., 2015). The psychological intervention protocol was based on principles of psychoneuroimmunology and included psychoeducation for the disease, treatment, self-care, and immune system functioning, as well as relaxation sessions with guided imagery on the components of the immune system eliminating malignant cells.

The primary endpoint of the study, where the PNI intervention was directed to, was the immune function estimated by counting lymphocyte subpopulations and serum antibody concentrations. The secondary variables were the clinical evolution estimated by the duration of symptoms, the duration of the symptomatic pharmacological treatment, and the QoL index.

Materials and methods

Patients

The study was conducted at the Pediatric Specialty Hospital of Maracaibo, Venezuela, a hospital specializing in the treatment of children with cancer. The research protocol was approved by the Bioethics Committee of the Hospital of Pediatric Specialties of Maracaibo (Venezuela) and in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments with human beings. This study's objectives and protocol were explained to the patient and her or his parents, and an informed consent was signed prior to the initiation of the study. We selected 30 out of 262 patients between 2013 and 2015 based on the following inclusion criteria: age range of 5–15 years, diagnosis of ALL, submitted to remission induction chemotherapy (based on the Berlin–Frankfurt–Münster protocol (BFM) for ALL) in the hospital, and without previous treatment for cancer. Exclusion criteria included learning disability, decreased visual acuity, neurological impairment, and other comorbidities or additional genetic syndromes.

We recruited a cohort of 12 patients (50% female) from 2013 to 2014 that received psychoeducation in relation to their treatment and disease, which served as control group and a cohort of 18 patients (39% female) from 2014 to 2015 that additionally received psychological intervention based on the principles of psychoneuroimmunology (PNI), which served as the PNI group (Figure 1). Due to the nature of the interventions, masking was not applicable. Besides, we did not use a randomized group assignment because the hospital infrastructure did not allow keeping the control group separated from the PNI group as to prevent control patients from being aware of the PNI material, which would have biased the results and considered bioethically unacceptable.

Chemotherapy

Induction to remission is the first phase of the BFM protocol, with a duration of approximately 64 days. The aim is to produce a complete remission of ALL, which is obtained when the percentage of blasts is less than 5 percent blasts with normal or slightly diminished cellularity and with signs of recovering hematopoiesis. The pharmacological regimen was customized based on the immunophenotype and the risk category (standard, intermediate, and high) of

each patient, and included vincristine, cardioxane, daunoblastine, asparaginase, prednisone, cyclophosphamide, and mercaptopurine.

Psychosocial support intervention

This intervention consisted of different activities organized to ensure optimal learning of children about the disease and medical treatment. The first author (J.C.F.) designed a structured intervention, aimed at children between 5 and 15 years of age diagnosed with leukemia. For specific psychoeducation on immune function, a didactic tale entitled "A battle won, my fight against leukemia" was written (Chacín de Fernández, 2014c). This story is organized into four chapters: (1) Knowing my body, (2) Now I know what is happening to me, (3) My treatment, and (4) The power of my mind. An activity notebook was also developed by J.C.F. (Chacín de Fernández, 2014b) to verify concepts dynamically learned and consisted of activities that motivated and reinforced the knowledge that the patient had to get for proactively coping with the disease and successfully complete the treatment. In addition, the intervention was complemented by playful strategies, using puppets and posters representing their immune system and memory games related to the psychoeducational story "A battle won, my fight against leukemia." J.C.F. wrote a script for relaxation and guided imagery based on psychoneuroimmunology in order to promote the child's imagination toward the healing process (Chacín de Fernández, 2014a). The tale was of interest to all patients, as they had the opportunity to read it in print or see an animated narration on a computer screen. However, given the differences in age, puppets were used for the illustrative explanation of the immune system to children from 5 to 10 years and sheets and memory sets for the older ones.

The intervention was applied by trained psychologists, in 30-minute sessions, and was attended by both the child and their parents or representatives, either in the hospital or in the outpatient clinic. The intervention was implemented in two phases: (1) the first phase covered from days 1 to 33 of the chemotherapy protocol, and consisted of emotional support and general psychoeducation on the disease and medical treatment for both groups and a specific psychoeducation on the immune system only for the PNI group; (2) the second phase covered from days 33 to 64 of the chemotherapy protocol involved the continuation of emotional support for both groups, and additionally, daily relaxation sessions and guided imagery specific to the immune function and its influence on the disease only for the PNI group. The children showed a receptive attitude to the applied therapy.

Clinical data collection

To evaluate the disease process in children, immunological, clinical, and pharmacological parameters were considered. Patient's symptoms and signs, administered medications by therapeutic categories, supportive therapies, as well as clinical complications, were recorded daily during hospitalizations.

Immune evaluation

Patient immunity was assessed during chemotherapy by counting natural killer (NK) cells and T lymphocyte subpopulations (innate and cellular immunity markers, respectively) and determination of serum concentrations of antibodies and B lymphocytes (cellular immunity markers). Venous blood samples were collected from each subject at days: 1 (prior to treatment), 33, and 64 (the culmination of the induction) of the chemotherapy protocol. The subpopulations of total lymphocytes, NK cells, T lymphocytes (total, CD8+, and CD4+), and B cells were measured in heparinized blood samples by flow cytometry (Ulrich et al., 2008) using fluorescently labeled monoclonal antibodies against surface activation markers: anti-CD56-PE, anti-CD16-FITC, anti-CD3-PE, anti-CD8-FITC, anti-CD4-perCP, and anti-CD19-APC. However, immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) concentrations were estimated in serum samples by immunoturbidimetry as previously described (Ramos et al., 2004).

QoL assessment

QoL was assessed using the QoL Questionnaire in Pediatric Oncology (Bernabeu Verdú, 2003) that was validated and modified for a Venezuelan sample with the evaluation of each question by experts in the field of oncology and psycho-oncology. A Cronbach's $\alpha = 0.717$ indicated that the instrument had internal consistency and scale reliability. The instrument was administered three times: on day 1 (before the start of induction), day 33, and day 64 (end of induction) of the chemotherapy.

Statistical analysis

The results of 26 of 30 pediatric patients who concluded the interventions were analyzed, excluding 4 (2 out of each group) who died during the study due to complications associated with the chemotherapy and medical pre-conditions (Figure 1). Data were expressed as mean ± standard error or 95 percent confidence interval (95% CI). The difference between two frequencies was assessed using the Chi-square test or Fisher's exact test. The difference between two averages was evaluated using the appropriate Student's t parametric test or the non-parametric Mann–Whitney U test. The comparison among three or more means was done by one-way analysis of variance (ANOVA) for an independent variable and two-way ANOVA for repeated measures for two independent variable variables

Table 1. Accumulative duration of the most frequent signs, symptoms, and clinical complications during hospitalizations.

	Controls (mean ± s.e.m. days), $N = 10$	PNI (mean ± s.e.m. days), $N = 16$
Signs and symptoms		
Fever	16.0 ± 6.7	2.7 ± 0.8*
Cutaneous pallor	12.2 ± 2.4	9.9 ± 1.9
Pain	11.5 ± 2.8	4.6 ± 1.0*
Hepatosplenomegaly	5.5 ± 2.4	6.7 ± 2.0
Adenopathy	4.6 ± 2.2	3.2 ± 1.1
Hematomas	2.1 ± 1.1	2.6 ± 1.1
Hiporexia	1.7 ± 0.8	1.5 ± 0.4
Bleeding	1.6 ± 0.6	0.8 ± 0.6
Emesis	1.5 ± 0.9	0.4 ± 0.2
Complications		
Neutropenia	22.5 ± 5.1	13.7 ± 3.1
Trombopenia	19.8 ± 4.7	17.6 ± 2.7
Anemia	13.3 ± 3.8	14.0 ± 2.7

PNI: psychoneuroimmunology-based intervention.

Mean of the continuous and non-continuous days during which the clinical event was present.

*Significant difference from controls (for fever: $p = 0.0201$; for pain: $p = 0.0116$; two-tailed Student's t-test).

followed by an appropriate multiple comparisons test (Holm–Sidak's and Bonferroni's, respectively). The degree of association between the dependent variables was determined using Spearman's non-parametric correlation analysis. Unless otherwise noted, tests were two-tailed and statistical significance was established either by non-overlapping 95 percent CI or p-value < 0.05.

Trial registration

The study was registered with LOCTI (www.locti.co.ve), as "Protocolo de intervención psico-social al niño con cáncer, fundamentado en la psiconeuroinmunología," repository #2906.

Results

There were no significant between-group differences respect to age (controls: 9.8 ± 1.2 years; PNI = 10.1 ± 0.9 years; mean ± s.e.m.), socioeconomic strata (lower strata: control: 80%, PNI: 66%; Graffar scale), and family structure (nuclear-type family, a couple and their children: control 50%, PNI 56%; the rest were single-parent families or reconstructed families). According to BFM protocol criteria (Salgado et al., 2015), 75 percent of the PNI group were at high risk (intermediate and standard risk: 18.75% and 6.25%, respectively), whereas only 40 percent of controls were at high risk (intermediate risk: 60%), but this difference was not statistically significant ($p = 0.1087$; Fisher's

exact test). The average percentage of blasts was similar in both groups at the start of induction chemotherapy: controls = 66 percent (95% CI: 46%, 86%), PNI = 57 (95% CI: 43, 71). Blasts were practically absent (range: 0%–0.4%) in both groups at days 33 and 64 of chemotherapy.

Hospitalization during induction chemotherapy

The controls had a mean of 2.30 hospital admissions (95% CI: 1.34, 3.3) and hospitalization duration of 33 days (95% CI: 21, 46 days), whereas the PNI group had 1.7 admissions (95% CI: 1.3, 2.0) and 24 days of hospitalization duration (95% CI: 17, 31). These between-group differences did not reach statistical significance ($p = 0.1324$, $t = 1.558$). The mean duration of chemotherapeutic induction for both groups was similar, 85 days (95% CI—controls: 75, 96; PNI: 77, 93). Of note, the induction phase lasts 64 days according to the ALL IC-BFM protocol, but it is usually extended if necessary by the clinical condition of the patient.

Symptoms, signs, and complications during hospitalizations

Table 1 shows the duration of the most frequent symptoms and clinical complications during hospitalizations. In the PNI group, the mean duration of fever and pain were 13 ± 5 and 7 ± 3 days, respectively, shorter than in controls (fever: $p = 0.0201$, $t = 2.4895$; $p = 0.0116$, $t = 2.7348$; $df = 24$; two-tailed t-test). The mortality rate was similar in both groups (control: 17% vs PNI: 11%).

Use of medications and supportive therapy

Table 2 shows the duration (in days) of symptomatic treatments and more frequent support therapies during hospitalizations. The administration of antibiotics, pain therapy, and antipyretics were 20 ± 10, 13 ± 3, and 13 ± 6 days, respectively, shorter in the PNI group than in controls (antibiotics: $p = 0.0522$, $t = 2.0425$; pain therapy: $p < 0.0001$, $t = 4.9984$; antipyretics: $p = 0.0286$, $t = 2.3294$; $df = 24$, two-tailed Student's t-test).

Cellular immunity parameters

The mean number of total lymphocytes was below the normal reference range (2000–2700 per μL) in both groups across all measurements (Figure 2(a)). The two-way ANOVA did not reveal significant global differences between the PNI and control groups (intervention: $F(1, 71) = 0.070$, $p = 0.7922$; time: $F(2, 71) = 1.436$, $p = 0.2447$; interaction: $F(2, 71) = 1.678$, $p = 0.1940$). However, the total number of lymphocytes was 53 percent

Table 2. Accumulative duration of the most frequent pharmacological symptomatic treatments and supportive therapies treatments during hospitalizations.

	Controls (mean ± s.e.m. days), $N = 10$	PNI (mean ± s.e.m. days), $N = 16$
Symptomatic treatment		
Antibiotics	37.4 ± 11.5	17.4 ± 3.0*
Pain therapy	17.5 ± 2.7	4.9 ± 1.1*
Antipyretics	16.1 ± 7.0	3.0 ± 0.9*
Antiemetics	1.8 ± 0.7	2.6 ± 0.7
Support therapies		
Platelets transfusion	6.9 ± 1.8	7.7 ± 2.8
Colony stimulating factor	4.2 ± 2.3	3.6 ± 1.5
RBC transfusion	2.7 ± 0.7	3.0 ± 0.8
Respiratory therapy	1.9 ± 0.9	0.4 ± 0.4

PNI: psychoneuroimmunology-based intervention; RBC: red blood cell.
Mean of the continuous and non-continuous days during which the clinical event was present.
*Significant difference from controls ($p < 0.05$).

Figure 2. Comparison of the average number of circulating lymphocytes and NK cells between control and PNI patients before (day 1) and during (days 33 and 64) the induction chemotherapy. Each point represents mean ± s.e.m. Horizontal dotted lines depict normal reference ranges: total lymphocytes = 2000–2700 per μL; NK cells = 200–300 per μL. (a) Total circulating lymphocytes (includes all subtypes; *significant difference of PNI respect to controls ($p < 0.05$, Student's t-test)). (b) Circulating NK cells (*significant difference of PNI respect to controls ($p < 0.05$, two-way ANOVA followed by Newman–Keuls test)).

higher in the PNI group compared to controls at day 64 ($p = 0.0028$, $t = 3.328$, $df = 24$, Student's t-test).

The mean number of NK cells was above the normal reference range (200–300 per μL) in the PNI group, while it was within that range in controls except on day 64 when it was below the range (Figure 2(b)). Two-way ANOVA showed a significant overall between-group difference, specifically at day 64, when NK cells in the PNI group were higher than the mean value of controls (intervention: $F(1, 69) = 11.88$, $p = 0.0010$; time: $F(2, 69) = 0.1953$, $p = 0.8230$; interaction: $F(2, 69) = 1.333$, $p = 0.2703$).

The mean number of T lymphocytes was below the normal reference range (1400–2000 per μL) in both groups across all measurements (Figure 3(a)). Two-way ANOVA did not reveal any overall difference between the PNI group and controls regarding the average number of T lymphocytes (intervention $F(1, 71) = 0.00006$, $p = 0.9937$; time: $F(2, 71) = 1.240$, $p = 0.2956$; interaction: $F(2, 71) = 1.331$, $p = 0.2706$).

The mean number of CD4+ T lymphocytes of the PNI group was within or close to the lower limit of the normal reference range (700–100 per μL) in all three measurements performed during chemotherapy (Figure 3(b)). In contrast, CD4+ T lymphocytes in controls were below the normal reference range and significantly lower than the mean of the PNI group ($p = 0.0005$, $t = 4.018$, $df = 24$; unpaired t-test). Yet, two-way ANOVA did not reveal overall between-group differences in CD4+ T lymphocytes, suggesting that an effect only occurs at the end of the PNI intervention (intervention: $F(1, 71) = 0.01636$, $p = 0.8986$; time: $F(2, 71) = 2.241$, $p = 0.1138$; interaction: $F(2, 71) = 1.885$, $p = 0.1594$).
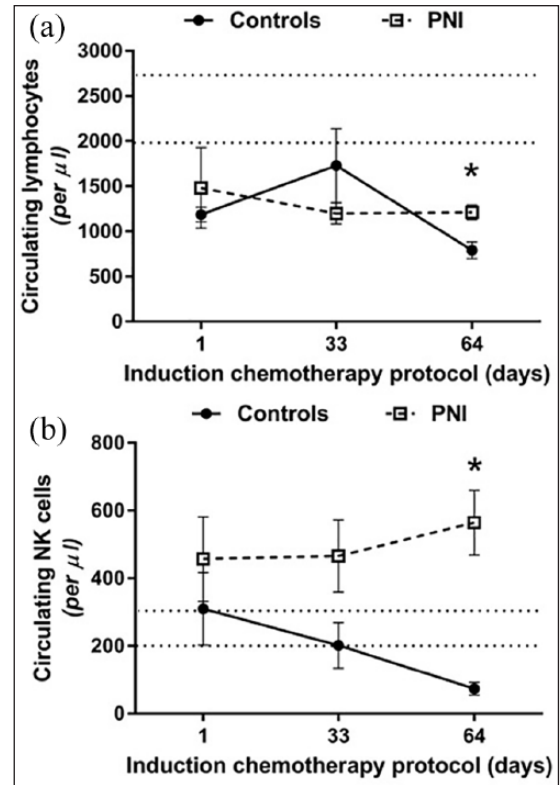
The average number of CD8+ T lymphocytes at days 1 and 33 was around the lower limit of the normal reference range (600–900 per μL) in both groups (Figure 3(c)). CD8+ T lymphocytes at day 64 remained within the normal reference range in the PNI group, while in controls significantly decreased below this range to 50 percent of the mean value of the PNI group ($p = 0.0006$, $t = 3.957$, $df = 24$, unpaired t-test). Two-way ANOVA did not reveal overall between-group differences in CD8+ T lymphocytes, suggesting that an effect only occurs at the end of the PNI intervention (intervention: $F(1, 71) = 1.894$, $p = 0.1730$; time: $F(2, 71) = 0.3318$, $p = 0.7187$; interaction: $F(2, 71) = 1.250$, $p = 0.2926$).

Mean CD4+ T/CD8+ T ratios were within the normal reference range (CD4T/CD8T: 1.1–1.4) at day 1, but it decreased to levels below that range at days 33 and 64 of the chemotherapy protocols in both groups. Two-way ANOVA did not reveal any significant difference in
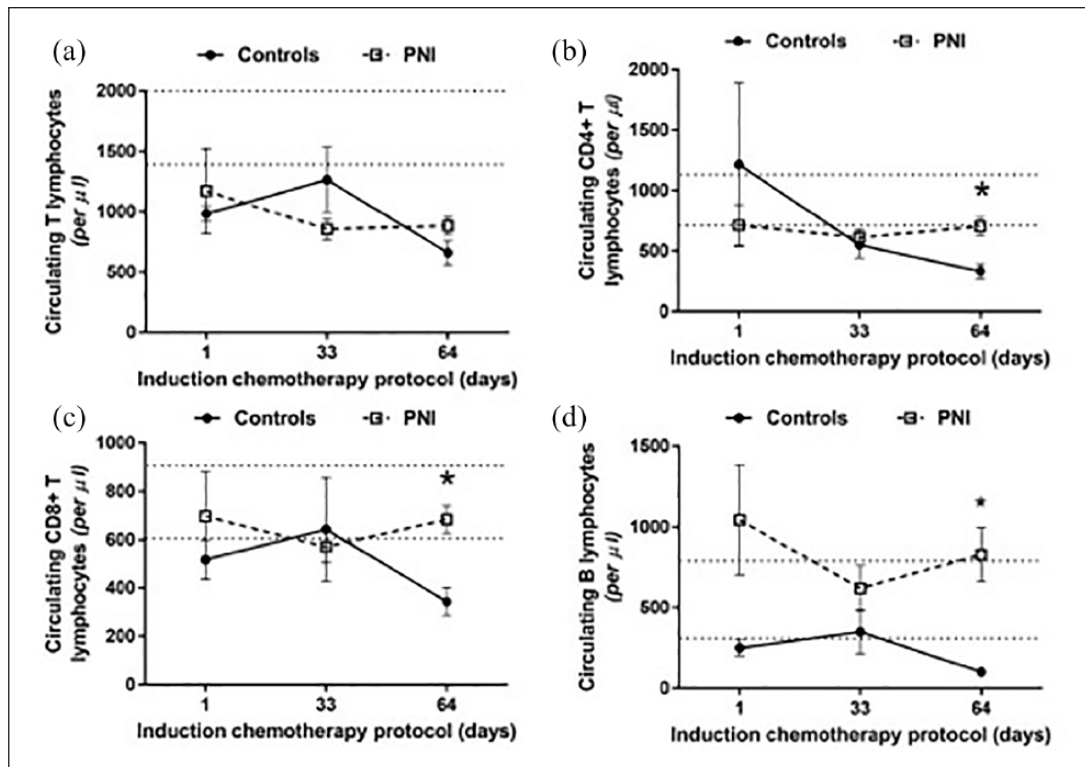
Figure 3. Comparison between the time course of the number of all T lymphocytes, subpopulations of T lymphocytes, and B lymphocytes. Each point represents mean ± s.e.m. Horizontal dotted lines depict normal reference ranges: (a) T lymphocytes range = 1400–2000 per μL. (b) CD4+ T lymphocyte range = 700–1100 per μL (*significantly higher in the PNI group than in the controls ($p < 0.0001$, unpaired t-test)). (c) CD8+ T lymphocytes range = 600–900 per μL (*significantly higher in the PNI than in the controls ($p < 0.0001$; unpaired t-test)). (d) B lymphocytes range = 300–800 per μL (*significantly higher in the PNI group than in the controls ($p < 0.001$; Mann–Whitney U test)).

CD4+ T/CD8+ T ratios neither between groups nor over time (intervention: $F_{(1, 71)} = 0.2367$, $p = 0.6281$; time: $F_{(2, 71)} = 2.521$, $p = 0.0876$; interaction: $F_{(2, 71)} = 0.7066$, $p = 0.4968$).

The mean number of B lymphocytes was approximately around the normal reference range (300–800 per μL) at days 1 and 33 in both groups (Figure 3(d)). The mean number of B lymphocytes at day 1 seemed to be higher in the PNI group than in the controls. Yet, this apparent large difference did not reach statistical significance ($p = 0.2232$, $U = 50$; Mann–Whitney U test) due to the very high variance seen in the PNI group (1044 ± 342 per μL; 95% CI: 317, 1772), but not in control group (250 ± 49 per μL; 95% CI: 136, 364). Yet, two-way ANOVA revealed marked between-group difference (intervention: $F_{(1, 71)} = 11.06$, $p = 0.0014$; time: $F_{(2, 71)} = 0.4068$, $p = 0.6673$; interaction: $F_{(2, 71)} = 0.8446$, $p = 0.4340$). B lymphocytes at day 64 were significantly higher in the PNI group (828 ± 667 per μL; 95% CI: 472, 1183) than in controls (100 ± 54 per μL; 95% CI: 62, 139) ($p = 0.0002$, $U = 14$, Mann–Whitney U test).

Humoral immunity parameters

Mean serum IgG concentrations were within the normal reference range (700–1600 mg/dL) in both groups throughout the induction of chemotherapy (Figure 4(a)). Two-way ANOVA did not reveal any significant between-group difference in IgG (intervention: $F_{(1, 71)} = 0.7978$, $p = 0.3748$; interaction: $F_{(2, 71)} = 0.002307$, $p = 0.9977$) although there was a significant decrease in IgG over time (time: $F_{(2, 71)} = 3.143$, $p = 0.0492$).

Mean serum IgM concentrations were above the normal reference range (40–260 mg/dL) in the PNI group over time, but within the range in controls (Figure 4(b)). Two-way ANOVA indicated that IgM in the PNI group was significantly higher than in controls (intervention: $F_{(1,57)} = 7.348$, $p = 0.0089$; time: $F_{(2, 57)} = 0.4181$, $p = 0.6603$; interaction: $F_{(2, 57)} = 0.3329$, $p = 0.7182$), specifically at days 1 and 64 ($p = 0.0525$, $U = 16.5$ and $p = 0.008$, $U = 9.00$; respectively; Mann–Whitney U test).

Mean serum IgA concentrations were within the normal reference range (70–400 mg/dL) across all measurements (Figure 4(c)). However, IgA was significantly higher in the PNI group than in the controls at day 64. Two-way ANOVA
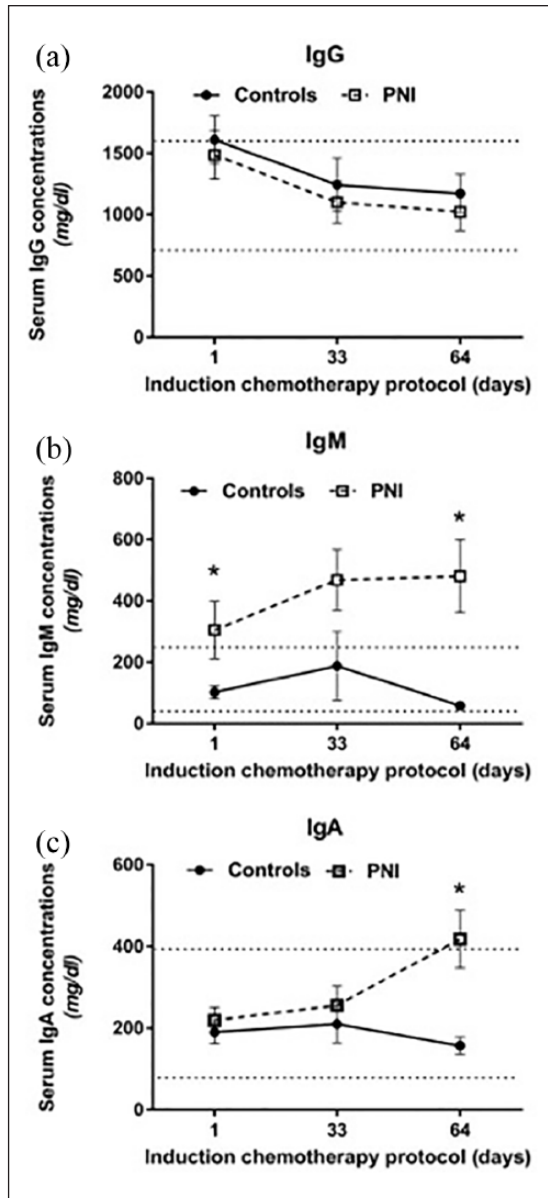
Figure 4. Average serum antibody subtypes during chemotherapeutic induction in controls and patients undergoing PNI. Each point represents mean $\pm$ s.e.m. Horizontal dotted lines depict normal reference range (IgG=700–1600 mg/dL; IgM=40–260 mg/dL; IgA=70–400 mg/dL). (a) IgG antibodies. (b) IgM antibodies (*significantly overall higher serum IgM concentrations in PNI group respect to controls ($F(1, 57)=7.348$, $p=0.0089$, two-way ANOVA)). (c) IgA antibodies (*significantly overall higher serum IgA concentrations in PNI group respect to controls: $F(1, 71)=6.981$, $p=0.0101$, two-way ANOVA and significant intervention $\times$ time interaction: $F(2, 71)=3.151$, $p=0.0489$, two-way ANOVA).

confirmed an overall between-group difference in IgA and also revealed a distinct patterns of concentrations changes over time for each group (intervention: $F(1, 71)=6.981$, $p=0.0101$; time: $F(2, 71)=1.329$, $p=0.2714$; intervention $\times$ time: $F(2, 71)=3.151$, $p=0.0489$).

QoL

The average QoL index and the proportions of patients by category (high-medium vs low) were similar in both groups at the beginning of induction chemotherapy (for indexes: $p=0.0146$, $t=2.640$, $df=23$, unpaired t-test; for proportions: $p=0.3644$, Fisher's exact test). Nevertheless, the QoL index increased significantly in the PNI group, but not in the control group; as a result, it was 14 percent higher in the PNI than the controls at day 64 (Figure 5(a)). Accordingly, the proportion of patients in the high or medium–high QoL categories was significantly higher in the PNI group (81%) compared to control group (33%) at day 64 (Figure 5(b)).

Correlations between relevant clinical features, immunity markers, and QoL

The immunological markers were linearly correlated with the duration of clinical, therapy outcomes, and QoL. Concretely, CD4+ T cells negatively correlated with fever, pain therapy, antipyretics, antibiotics, and QoL. B lymphocytes, NK cells, and IgA negatively correlated with pain therapy, while IgM negatively correlated with fever and antibiotics. On the other hand, CD4+ T cells, CD8+ T cells, IgA, and IgM positively correlated with QoL (Table 3). The duration of symptoms was positively correlated with the duration of treatment: fever and pain with antibiotics, pain therapy and antipyretics ($p \leqslant 0.05$), whereas QoL index was inversely correlated with fever ($r=-.445$, $p=0.0292$).

Discussion

The PNI intervention had beneficial effects on immunological markers, the clinical course, and QoL of ALL patients, during remission induction chemotherapy, that cannot be ascribed to between-group differences in age, gender, family structure, socioeconomic status, initial immune status, level of risk, and duration of induction chemotherapy (Table 4).

Immunological and clinical outcomes

The immunological markers, number of NK, CD8+ T, CD4+ T, and B lymphocytes, as well as serum concentrations of IgM and IgA, were significantly higher in the PNI group compared to the controls upon completion of the chemotherapy protocol. This immunological profile could explain the shorter duration of fever, pain, and the administration of antibiotics, antipyretics, and pain in the PNI group compared with the controls since the immunity markers were linearly correlated with those clinical outcomes. Specifically, the duration of fever and administration of antibiotics were negatively correlated with CD4+ T cell numbers and IgM concentrations, while the duration of pain therapy was negatively correlated with IgA levels and
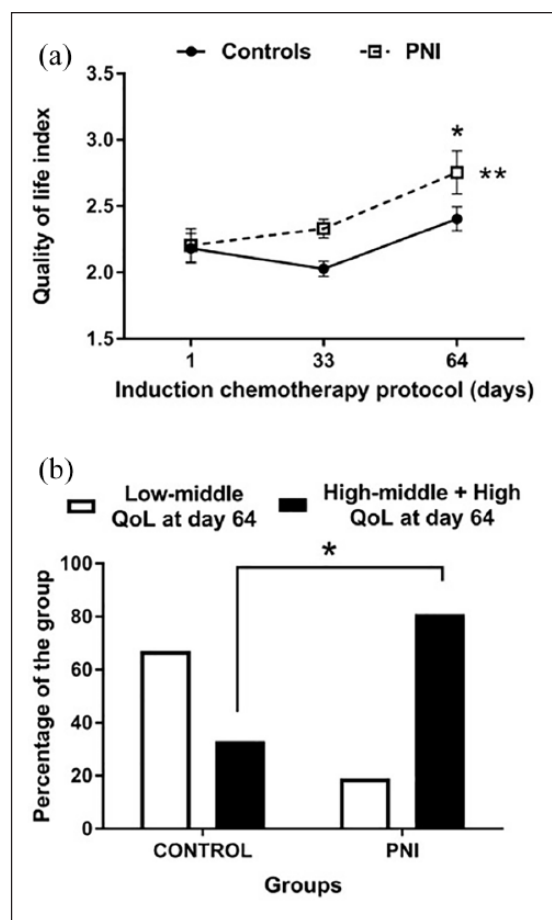
Figure 5. Quality of life at day 64 of induction chemotherapy in ALL pediatric patients subjected to PNI and conventional (control) interventions. (a) Quality of life index. Each point represents mean ± s.e.m. (*significant difference with respect to controls ($p < 0.01$; two-way ANOVA); **significantly higher than day 1 (for PNI) or day 33 (for controls) (PNI: $p < 0.01$; controls: $p < 0.05$; one-way ANOVA)). (b) Percentage by category of quality of life at day 64 (*significant difference ($p < 0.05$, Fisher's exact test)).

the number of CD4+ T, NK, and B cells. It is widely established that all of these immunological markers have key roles in immunity (Saraiva et al., 2012). NK cells are part of the innate immune system, and although they do not destroy pathogens directly by phagocytosis, they destroy infected or neoplastic cells. However, lymphocytes mediate acquired immunity. Specifically, CD4+ T lymphocytes act primarily against parasites, bacteria, and fungi, and to a lesser extent against viruses and tumor antigens; CD8+ T lymphocytes act primarily against tumor cells and viruses; and B lymphocytes produce immunoglobulin antibodies. IgM antibodies contribute to eliminating antigens by coating them (opsonization) for the fixation of complement, while the secretory IgA antibodies protect the mucosal surfaces from toxins, viruses, and bacteria by means of direct neutralization or by prevention of the attachment to the surface of the mucosa (Schroeder and Cavacini, 2010). Therefore, it is possible to postulate that the improvement in the cellular (innate or acquired) and humoral immune functions could be associated with the PNI intervention, and consequently, with a curtailment in the inflammatory-infectious processes and their associated symptoms, fever, and pain. This would be consistent with the reduction in the duration of treatments.

Improvement of immunity after psychological interventions has been reported. Psychotherapeutic techniques designed to facilitate personal growth, interpersonal relationships, and coping lead to an increase in the number and activity of NK cells in women who have suffered a loss of someone close to breast cancer (Bower et al., 2003). Relaxation and meditation training are associated with increased activity and numbers of NK cells in HIV-infected individuals (Robinson, 2002) and increased production of IL-4 and IL-10 anti-inflammatory cytokines of lymphocytes by NK cells in cancer patients (Carlson et al., 2003). Stress-reducing interventions that include relaxation and visualization increase secretory IgA in a manner dependent of

Table 3. Correlation matrix of statistically significant variables of the study.

	Immunity						
	NK cells	CD4T	CD8T	B cells	IgG	IgM	IgA
Clinical							
Fever	−0.344	−0.414*	−0.264	−0.160	0.307	−0.576**	−0.394
Therapy							
Antibiotics	−0.398	−0.537**	−0.384	−0.335	0.232	−0.543*	−0.402
Pain therapy	−0.586**	−0.588**	−0.317	−0.488*	−0.066	−0.404	−0.514*
Antipyretics	−0.343	−0.410*	−0.267	−0.227	0.290	−0.567	−0.371
Quality of life	0.345	0.452*	0.526*	0.398	−0.218	0.488*	0.503*

NK: natural killer.
*$p \leqslant 0.05$, two-tailed.
**$p \leqslant 0.01$, two-tailed.

Table 4. Summary of the significant changes associated with PNI-based psychological intervention on ALL pediatric patients.

Duration of signs and symptoms	
Fever	↓
Pain	↓
Duration of administration	
Pain therapy	↓
Antibiotics	↓
Antipyretics	↓
Immunity	
Natural killer (NK)	↑
B lymphocytes	↑
CD4+ T lymphocytes	↑
CD8+ T lymphocytes	↑
IgM antibodies IgA	↑
IgA antibodies	↑
Quality of life	↑

PNI: psychoneuroimmunology-based intervention; ALL: acute lymphocytic leukemia; IgM: immunoglobulin M; IgA: immunoglobulin A.

individual's personality (Valdimarsdottir and Stone, 1997). Cognitive coping strategies, relaxation, and social support may lead to changes in IgG and CD4+ T lymphocytes in HIV-infected patients (Antoni, 2003). Psychotherapeutic interventions may also influence the interaction of CD4+ T cells and B cells to change in their antibody production profile from IgM to IgG and IgA during an immune response (Oxenius et al., 1998). Noteworthy, we observed that a higher number of CD4+ T cells correlated with higher concentrations of IgA and IgM.

This study is the first to evaluate the efficacy of a PNI-based psychological intervention in pediatric leukemia patients and demonstrates an improvement in immunity that is clearly associated with shorter duration of symptoms and treatment, as well as an increase in the QoL. Improved immunity associated with PNI-based interventions in children has been reported only in the context of non-neoplastic diseases. For example, PNI-based psychotherapeutic intervention is associated with a high number of NK cells and a reduction in the IgE response against allergens in asthmatic children (Castés et al., 1999).

Effect on QoL

QoL also improves in ALL patients during induction-to-remission chemotherapy (Castillo-Martínez et al., 2009). However, we observed that an increase in the QoL index was significantly higher in the PNI group than in the controls. The combination of psychoeducation, relaxation technique-guided imagery, and cognitive therapy significantly diminishes stress and increases QoL in patients with head and neck, breast, and lung cancers (Barre et al., 2018). This improvement could be associated with less severe clinical symptoms and a higher immune function since QoL

was correlated negatively with duration of fever and positively with CD8+ T and CD4+ T cell count, as well as with IgM and IgA concentration. In support, improvement of some immunity markers along with QoL has been reported in women with cervical cancer (Nelson et al., 2008).

The psychological intervention protocol based on PNI in the context of psychosocial interventions

Previous studies have observed that psychosocial interventions improve immunological markers, and this improvement is positively correlated with psychological aspects (e.g. reduction of anxiety and negative mood, better coping, and habits), but without any enhancement in functioning status, symptomatology, and laboratory values (Andersen et al., 2004, 2007; Fawzy et al., 1990). A recent systemic review reported that the combination of progressive muscle relaxation and guided imagery decreases nausea and vomiting and improves psychological state in breast cancer patients, but the biological mechanisms are not known (Kapogiannis et al., 2018). In this study, we found in the patients subjected to the PNI intervention a close correlation between the increase of several key immune markers and a more satisfactory evolution of various clinical aspects of the disease, symptomatic treatment, and QoL. This PNI intervention differed from the psychosocial control protocol only in two activities, psychoeducation and guided imaginary related to immunology. This difference suggests that the presence of a PNI component in the intervention is somehow associated with the beneficial outcomes of this study, and it might represent an improvement over psychotherapeutic interventions lacking of this immunological aspect. Unfortunately, only limited evidence supports the influence of psychological conditioning on the immune (Cohen et al., 1994; Giang et al., 1996), and therefore, the mechanisms mediating this association remain to be elucidated.

Limitations and final considerations

The major limitation of the study was sample size and lack of stratified randomization by risk level. Therefore, the results are preliminary and cannot yet be generalized, but they justify future studies. However, the observed beneficial effects might not be due to the semantic framework of the PNI intervention, but other components, such as the relaxation training that reduces stress. In this regard, stress can facilitate the progression of cancer metastases by sympathetic activation of β-adrenoreceptor and cyclooxygenase 2 along with the associated inflammatory response (Ricon et al., 2019). Future studies should be designed to address these uncertainties, including the physiological mechanisms underlying the PNI intervention. Beyond these limitations, PNI interventions might empower patients with

psychological tools for modulating and improving their antitumor immune function and health status.

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