PIMS-TS Innate Cell Signature and Immunophenotype, Description and Comparison with a Cohort of Healthy Children, Kawasaki Disease, Severe Viral and Bacterial Infections.

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Research Article

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Abstract

A new clinical syndrome named as Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2 (PIMS-TS) has been described. This new disease is a main cause of hospital and pediatric intensive care unit (PICU). We made a prospective-retrospective observational study to describe the innate cell signature and immunophenotype of children admitted to PICU because of PIMS-TS (from March 2020 to September 2020). They were compared with previous cohorts of healthy controls and children admitted to PICU because bacterial infection, viral infection and Kawasaki disease (KD). Two hundred and forty seven children were studied: 183 healthy controls, 25 viral infections, 20 bacterial infections, 6 KD and 13 PIMS-TS. PIMS-TS showed the lowest percentage of lymphocytes and monocytes with higher relative numbers of CD4+ (p =0.000). Monocytes and neutrophils in PIMS-TS showed higher levels of CD64 expression (p = 0.000). Also, CD11a and CD11b were highly expressed compare to other severe viral or bacterial infections (p = 0.000). In conclusion, we describe and compare for the first time the innate cellular response of children with PIMS-TS with other severe forms of viral or bacterial infection and KD. These data should be further studied and may facilitate the diagnosis and management of these patients.

Background

A new type of affection temporarily linked to the new coronavirus SARS-CoV-2 has been described in childhood. This new clinical syndrome has been named as Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2 (PIMS-TS). It has clinical and analytical similarities to Kawasaki disease and suppose a main cause of hospital and pediatric intensive care unit (PICU) admission [1-3].

A majority of PIMS-TS cases are not related to active infections. There has been described the presence of immunity dysregulation or the release of autoantibodies. As treatment immunoglobulin and corticoids seem to have a preeminent role. The clinical response is usually quick with improvement in a short period of time[2, 4].

Related to the probable role of leukocyte dysregulation the study of the cellular response in PIMS-TS could be of interest. Also, its comparison with healthy children and other causes of PICU admissions may help to understand this new disease[5]. In this work we describe the innate cell signature and immunophenotype of children admitted to PICU because of PIMS-TS. Also, we compare it with healthy controls and children admitted to PICU because bacterial infection, viral infection and Kawasaki disease.

Material And Methods

Prospective-retrospective observational study conducted in a tertiary pediatric hospital after Ethics Committee for clinical research approval from Hospital Infantil Universitario Niño Jesús. Done in children admitted to PICU because of PIMS-TS from March 2020 to September 2020. At PICU admission a peripheral blood sample was extracted from a previously established intravenous line. It was done after parents or legal guardians consent. The volume obtained was 0.5 ml and collected in sterile EDTA tube. The sample handling was based on item 59 of the Spanish law on Biomedical Research. All methods were carried out in accordance with relevant guidelines and regulations.

Sample processing and analysis by flow cytometry

Samples were collected at room temperature or refrigerated at 4°C, used for CD45+ cells marking and analyzed by flow cytometry in a time period shorter than 24 hours. The antibodies were all from Biolegend®: CD45 (clone HI30), CD4 (clone OKT4), CD8 (clone SK1), CD64 (clone 10.1), CD11a (clone TS2/4) and CD11b (clone M1/70). The surface expression were measured by BD FACS Canto II flow cytometer (Becton Dickinson, New York, USA). Cells viability were confirmed by 7-AAD staining. At least 10,000 events were recorded for each sample. The flow cytometer settings and samples were prepared according to the manufacturer's instructions. Neutrophils, monocytes, and lymphocytes were identified on dot-plot profile and gated. The intensity of CD64 surface expression was measured as mean fluorescence intensity (MFI) in arbitrary units (monocytes as mCD64 and neutrophils as nCD64). The positive CD4, CD8, CD11a, CD11b and CD64 cells were expressed as percentage.

Cohorts of analysis

Four cohorts of analysis were described:

1. Healthy patients: individuals under the age of 18 who came for programed surgery. They do not present previous diseases or infection. Sample extraction was performed prior to intervention. Obtained in January 2018 to December 2019.
2. Viral and bacterial infections (VI and BI): individuals under 18 years of age admitted to PICU because of severe infection. Samples obtained at admission. Colonization was ruled out. Both groups are historical cohorts that have been described and published by our group before (see bibliography).
3. Kawasaki disease (KD): children admitted to hospital or PICU which Kawasaki disease criteria. Period January 2018-december 2019. The flow cytometry was performed at hospital admission and prior to the initiation of immunomodulatory medication.
4. PIMS-TS: children admitted to PICU which met criteria defined for this condition (see bibliography). To confirm SARS-CoV-2 infection a nasal and pharyngeal swab using real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) was used. The presence of SARS-CoV-2 IgG antibodies was studied through ELISA.

Statistical study

Statistical analysis was performed with the statistical program SPSS version 19.0 (IBM®). The quantitative values are expressed as mean and standard deviation. To compare quantitative variables between the bacterial and viral group a U Mann-Whitney test was used. Spearman's rank correlation coefficient
was bi-marginal calculated to measure the relationship between two continuous variables. A receiver operating characteristic (ROC) analysis with area under curve (AUC), sensitivity and specificity and cut-off values was performed for mCD64 and nCD64 to define its diagnostic accuracy for PIMS-TS. The cut-off values were calculated by Youden index. Findings of two-tailed \( p < 0.05 \) were considered statistically significant.

**Results**

A total of 247 children were included: 183 healthy controls, 25 viral infections, 20 bacterial infections, 6 Kawasaki disease and 13 PIMS-TS. In the PIMS-TS group 5 children were RT-PCR positive, 7 were Ig G positive and one RT-PCR and IgG positive at diagnosis. There were sex differences only in KD group. There were observed differences in age (Table 1). A negative correlation for lymphocytes with age was observed (\( r = -0.240, p = 0.001 \)). The CD64 expression in monocytes and neutrophils was not correlated with age or influenced by sex. The percentage of leukocytes and MFI of each type of leukocytes are described in table 1 and Figure 1. The significative differences between PIMS-TS and other groups are showed in Figure 1.

**CD64 and CD11a neutrophils expression utility as a PIMS-TS biomarker**

To study the usefulness of CD64 surface expression as a tool to predict PIMS-TS we evaluated the receiver operating characteristics curve. As seen in Figure 2 the mCD64, nCD64 and nCD11a areas under the curve (AUC) were near to 1. For mCD64 it was 0,994 (\( p=0,000 \); with a cut point of 29098 MFI and 94,1% specificity and 100% sensitivity). For nCD64 it was 0,992 (\( p=0,000 \); with a cut point of 8753 MFI and 90% specificity and 100% sensitivity). The nCD11a AUC was 0,992 (\( p=0,000 \); with a cut point of 5203 MFI and 91,7% specificity and 94,1% sensitivity).

**Discussion**

This paper compares for the first time the innate cellular signature and immunophenotyping of severe PIMS-TS with a large cohort of healthy control, other severe infections diseases and KD. We observed differences in almost all leukocyte populations. The most visible of these differences affect lymphocytes and monocytes, which have the lowest values in PIMS-TS. At the same time, we describe a differential expression of CD64, CD11a and CD11b. These leukocyte surface proteins were exceptionally high in PIMS-TS in monocytes and neutrophils.

The distribution of leukocyte populations is strikingly different in lymphocytes (Table 2 and Figure 1). PIMT-TS showed the lowest percentage with higher relative numbers of CD4+. This lymphopenia has already been described in adult population and severe critical children because of SARS-CoV-2[2, 6]. Compare to other severe viral infections, we observed that there was also a low percentage of monocytes and neutrophils (Figure 1). It is known that PIMS-TS is usually not associated with active SARS-CoV-2 infection. An immune dysfunction has been proposed as cause. Monocytes, neutrophils and lymphocytes are critical cells in viral first response. Their migration to infected tissues added to the SARS-CoV-2 capacity to dysregulate this response may cause this low cell count in peripheral blood[5]. Our group has previously described an increase CD18/CD11a complex (LFA-1) in leukocytes expression in two short series of PIMS-TS[7, 8]. It is congruent with an increased cellular predisposition to leave the bloodstream.

Concerning immunophenotyping, we should highlight the findings observed about CD64, CD11a and CD11b. The PIMS-TS showed higher CD64 expression compared to all groups. The CD64 indirectly reflect cytokine expression. As shown in Figure 2 the CD64 levels are even higher in PIMS-TS than in severe bacterial infections and KD. This CD64 expression may inform about a hyperinflammatory status[6, 7]. Compared to KD we observed differences in monocytes but not in neutrophils. The study of CD64 and CD11a expression could help in the differential diagnosis of PIMS-TS (Figure 2).

We also examined the percentage of CD11a and CD11b positive cells. We observed that both proteins were higher in neutrophils and monocytes than in viral or bacterial infections (Figure 1). Also, they were higher than KD but without significate differences. Related to CD11a, the MFI was also higher in neutrophils of PIMS-TS cases. In adults, the inflammation in the basis of SARS-CoV-2 showed a predominant presence of macrophages and neutrophils in the affected territory. In PIMS-TS this increased CD11a expression could be a sign of trafficking. These findings, added to the previously commented, are congruent with an inflammatory process and the trend of these cells to leave the bloodstream. This add interesting about the utility of anti-inflammatory drugs as a cornerstone in the management of these children[9-12].

This work has limitations. We observed age differences between the groups (Table 1). The distribution of leukocyte populations is influenced by this. The cohort’s internal homogeneity and the absence of correlation with age or sex in CD64, CD11a and CD11b expression may minimize this limitation. We did not study the relationship between the observed data and PIMS-TS clinical courses. This was not the aim of this work and should be considered in future works.

In conclusion, we describe and compare for the first time the innate cellular response of children with PIMS-TS with severe forms of viral and bacterial infection and KD. Our findings define a differential cell innate signature with presence of inflammation. These data should be further studied.

**Declarations**

a. This study was partially funded by "Fundación Alonso".

b. The authors have disclosed that they do not have any potential conflicts of interest.

c. The data will be available in case of request.

d. Collected through online web data manager. We use a custom code to anonymize.

e. Approved by the clinical research hospital committee.
f. Authors’ contributions: AGS wrote the paper and included the data. ILG co-wrote and corrected it, also helped in data inclusion and analysis. Each author participate in clinical care and patient’s inclusion. All authors except served as internal reviewers. AGS and MNM coordinated the work and appointed internal reviewers. ACR and MRO participated in the experimental area. All authors read and approved the final manuscript.

g. Consent to participate: All the cases included did so after the signing of the informed consent by parents / caregivers.

h. Consent for publication: The study participants were informed about the possibility of publication when signing the informed consent form. Also, all authors agree with the publication of the study.

References


Tables

Table 1. Age in months and sex of the cases included.
<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>N</th>
<th>Median (months)</th>
<th>Maximum (months)</th>
<th>Minimal (months)</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Male</td>
<td>93</td>
<td>66,19</td>
<td>332,77</td>
<td>2,35</td>
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<td>90</td>
<td>75,60</td>
<td>215,19</td>
<td>3,03</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td>13</td>
<td>1,84</td>
<td>11,71</td>
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<td>12</td>
<td>6,78</td>
<td>144,94</td>
<td>0,52</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>12</td>
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<td>197,58</td>
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<td>8</td>
<td>8,86</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>6</td>
<td>42,94</td>
<td>63,87</td>
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<td><strong>PIMS-TS</strong></td>
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</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>119,00</td>
<td>181,48</td>
<td>16,42</td>
<td></td>
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<tr>
<td>Female</td>
<td>6</td>
<td>98,15</td>
<td>172,03</td>
<td>28,42</td>
<td></td>
</tr>
</tbody>
</table>

$p = 0.000$; 0.000

Legend: MFI: mean fluorescence intensity, $p = \text{statistical signification.}$

**Table 2.** Comparison of leukocyte percentages and CD64, CD11a and CD11b expression. The mean fluorescence intensity for CD64 and CD11a on neutrophils and monocytes is also described and compared.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Virus</th>
<th>Bacteria</th>
<th>Kawasaki</th>
<th>PIMS</th>
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</thead>
<tbody>
<tr>
<td>% Neutrophils</td>
<td>38,0</td>
<td>34,00</td>
<td>10</td>
<td>13,10</td>
<td>40,80</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>65,4</td>
<td>89,0</td>
<td>9,2</td>
<td>36,0</td>
<td>76,2</td>
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<tr>
<td>%CD8</td>
<td>21,8</td>
<td>79,3</td>
<td>4,7</td>
<td>38,8</td>
<td>81,7</td>
</tr>
<tr>
<td>%CD4</td>
<td>19,0</td>
<td>57,3</td>
<td>4,0</td>
<td>20,0</td>
<td>73,3</td>
</tr>
<tr>
<td>Ratio CD4/CD8</td>
<td>1,32</td>
<td>96,3</td>
<td>0,0</td>
<td>2,13</td>
<td>3,98</td>
</tr>
<tr>
<td>% CD64+ Neutrophils</td>
<td>1,4</td>
<td>99,9</td>
<td>0,0</td>
<td>72,6</td>
<td>100,0</td>
</tr>
<tr>
<td>% CD11a+ Neutrophils</td>
<td>98,7</td>
<td>100,0</td>
<td>18,3</td>
<td>62,3</td>
<td>100,0</td>
</tr>
<tr>
<td>% CD11b+ Neutrophils</td>
<td>49,2</td>
<td>100,0</td>
<td>2,0</td>
<td>10,8</td>
<td>99,5</td>
</tr>
<tr>
<td>% CD11b/CD11a+ Neutrophils</td>
<td>48,4</td>
<td>99,2</td>
<td>2,2</td>
<td>1,2</td>
<td>98,0</td>
</tr>
</tbody>
</table>

**Immunophenotyping**

<table>
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<tr>
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<th>Bacteria</th>
<th>Kawasaki</th>
<th>PIMS</th>
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<tr>
<td>MFI CD64 Monocytes</td>
<td>4363,0</td>
<td>13776,0</td>
<td>1568,0</td>
<td>15391,5</td>
<td>37518,0</td>
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<tr>
<td>MFI CD64 Neutrophils</td>
<td>365,0</td>
<td>4355,0</td>
<td>146,0</td>
<td>139,0</td>
<td>51510</td>
</tr>
<tr>
<td>Ratio mCD64/gCD64</td>
<td>11,23</td>
<td>34,61</td>
<td>1,29</td>
<td>6,11</td>
<td>50,76</td>
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<tr>
<td>MFI CD11a Neutrophils</td>
<td>2585,0</td>
<td>8715,0</td>
<td>851,0</td>
<td>1091,0</td>
<td>591,0</td>
</tr>
</tbody>
</table>

Legend: MFI: mean fluorescence intensity, $p = \text{statistical signification.}$

**Figures**
Figure 1

Leukocyte populations and immunophenotyping of patients included in the study. From left to right in each of the graphs: 183 healthy controls, 25 viral infections, 20 bacterial infections, 6 Kawasaki disease and 13 PIMS-TS. An arrow indicates those populations showing significant differences with PIMS-TS patients. A. Percentage of leukocyte populations. B. The first row compares the percentage of neutrophils positive for CD64, CD11a and CD11b. The bottom row compares the mean fluorescence intensity for CD64 in monocytes and neutrophils.
The ROC curve for CD64 on monocytes, CD64 on neutrophils and CD11a on neutrophils is shown. The values of the area under the curve with statistical significance and confidence interval are indicated.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Signification</th>
<th>95% confidence interval</th>
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<tbody>
<tr>
<td>MFI CD64 Monocytes</td>
<td>0.994</td>
<td>0.000</td>
<td>0.986 - 1.000</td>
</tr>
<tr>
<td>MFI CD64 Neutrophils</td>
<td>0.992</td>
<td>0.000</td>
<td>0.983 - 1.000</td>
</tr>
<tr>
<td>MFI CD11a Neutrophils</td>
<td>0.979</td>
<td>0.000</td>
<td>0.956 - 1.000</td>
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Figure 2