Age-Related Lymphocyte Subset Changes in the Peripheral Blood of Healthy Children – a Meta-Study

Ulrich Sacka Fee Gerlinga Attila Tárnokb

a Department for Clinical Immunology and Transfusion Medicine,
b Department for Pediatric Cardiology, Cardiac Center Leipzig GmbH, University of Leipzig, Germany

Key Words
Flow cytometry · Immunophenotyping · Childhood · Lymphocytes

Summary
Background: Immunophenotyping is an important diagnostic tool for decision making in various diseases. Although clinical interpretation relies on measurable aberrations of the patient’s values from normal ranges, age-dependent are rarely available. Materials and Methods: The present study is aimed to combine published data about normal values of peripheral blood lymphocyte subpopulations to describe age-dependent changes from the neonate to the adult. These values could serve in a better way as normal values in comparison to present ones. Furthermore, this investigation allows us to define valid data even for short periods in children's life and to investigate the influence of technical approaches, sample preparation, antibody selection, measurement equipment, and data analysis. Results: Development-related alterations of lymphocyte subset counts in children could be extracted from the pre-existing papers for diagnostic use. These results were mostly independent from gender, ethnic factors, procedure of sample collection, anticogulation, pre-analytical procedures, time to workbench, applied method for immunophenotyping, staining procedure, selected monoclonal antibodies, technical devices, and software products. Conclusion: Our data indicate that previous normal values are not sufficiently precise for the interpretation of lymphocyte subsets in children. Mainly during the 1st year of life, count and subset distribution of lymphocytes is different from that of adults. Therefore, a close meshed data set of normal values is required to guarantee adequate diagnostic interpretation.

Schlüsselwörter
Durchflusszytometrie · Immunphänotypisierung · Kindheit · Lymphozyten

Zusammenfassung
Introduction

It is a well-known fact that developmental changes from the neonate to the adult massively influence the composition of peripheral blood leukocytes as well as that of the lymphocytes. Immunophenotyping is an important diagnostic tool for decision making in various diseases [1–6]. Typical examples are:

- diagnosis of immunodeficiencies [7],
- monitoring of systemic and chronic diseases [8–12],
- cellular diagnostics of allergy [13, 14],
- functional characterization of cells [15, 16],
- genetic investigation [17, 18],
- differential diagnosis of lymphocytosis [19],
- diagnosis of leukemia [5, 20–24],
- investigation of T-cell receptor expression in Kawasaki syndrome [25–26],
- investigation of pregnancy disturbances [27],
- quality control in hemotherapy [28–30].

Furthermore, there are several indications that are presently not so important for routine diagnostics [31].

Clinical decision making relies on measurable aberrations of the patient’s values from the ‘normal’ [32–34]. In children correct decision making heavily depends on the availability of these normal ranges for the respective age groups. It is of great concern that until today no reliable data exist that take into account the age-dependent changes. In many cases for children the normal values used for diagnostics are based upon data of adults. There is only a handful of published studies that report the lymphocyte subset composition of children in different age groups. These studies have several drawbacks, making their application difficult in everyday clinical diagnostic settings:

- limited number of children enrolled,
- different age groups or a limited age range investigated,
- technical equipment, namely flow cytometers and software products,
- reagents including lysis and antibodies,
- different parameters analyzed,
- one- or two-platform technologies.

Aim

The present study is aimed to combine existing studies (as far as they were combinable) into a meta-study describing age-dependent changes from the neonate to the adult. These values could serve in a better way as normal values as present ones. Furthermore, this investigation allows us to define valid data even for short periods in children’s life and to investigate the influences of technical approaches, sample preparation, antibody selection, measurement equipment, and data analysis.

Materials and Methods

The present work is a meta-analysis compiling data published earlier by others. For this study, we screened databases of all publications reporting of normal values in children. As the primary raw data were in most cases not at hand, all available data (single values and calculated values) were included. We tried to contact all authors of these publications in order to ask them for the primary data. Unfortunately, this was successful only in few cases. We included exclusively studies based on cytometry and staining with monoclonal antibodies (for more details on cytometry and applications in clinical diagnostics see [35]). Technical options such as isolation of lymphocytes prior to staining, simultaneous staining with multiple fluorescent dyes, or data analysis options were investigated for influence on data, but data from these studies were not excluded.

Table 1. Age-related lower and upper limits (calculated by mean ± SD) of lymphocyte counts in the peripheral blood

<table>
<thead>
<tr>
<th>Age</th>
<th>T helper cells</th>
<th>Cytotoxic T cells</th>
<th>T cells</th>
<th>B cells</th>
<th>NK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−1 SD</td>
<td>+1 SD</td>
<td>−1 SD</td>
<td>+1 SD</td>
<td>−1 SD</td>
</tr>
<tr>
<td>Newborn</td>
<td>1.50 ± 2.00</td>
<td>0.73 ± 1.65</td>
<td>2.10 ± 3.32</td>
<td>0.46 ± 0.94</td>
<td>0.48 ± 1.54</td>
</tr>
<tr>
<td>1 month</td>
<td>2.25 ± 4.27</td>
<td>0.86 ± 2.62</td>
<td>3.02 ± 5.94</td>
<td>0.93 ± 2.37</td>
<td>0.26 ± 0.84</td>
</tr>
<tr>
<td>3 months</td>
<td>2.27 ± 4.33</td>
<td>0.88 ± 2.58</td>
<td>3.17 ± 6.11</td>
<td>0.93 ± 2.37</td>
<td>0.26 ± 0.84</td>
</tr>
<tr>
<td>6 months</td>
<td>2.30 ± 4.52</td>
<td>1.10 ± 2.82</td>
<td>3.17 ± 6.11</td>
<td>0.93 ± 2.37</td>
<td>0.26 ± 0.84</td>
</tr>
<tr>
<td>1 year</td>
<td>1.71 ± 3.51</td>
<td>1.21 ± 2.57</td>
<td>1.87 ± 4.47</td>
<td>0.58 ± 1.92</td>
<td>0.14 ± 0.70</td>
</tr>
<tr>
<td>2 years</td>
<td>1.60 ± 3.14</td>
<td>1.06 ± 2.60</td>
<td>1.87 ± 4.47</td>
<td>0.58 ± 1.92</td>
<td>0.14 ± 0.70</td>
</tr>
<tr>
<td>3 years</td>
<td>1.18 ± 2.76</td>
<td>0.61 ± 1.79</td>
<td>1.84 ± 4.34</td>
<td>0.58 ± 1.92</td>
<td>0.03 ± 0.59</td>
</tr>
<tr>
<td>4 years</td>
<td>1.14 ± 2.08</td>
<td>0.54 ± 1.32</td>
<td>1.39 ± 3.47</td>
<td>0.18 ± 1.14</td>
<td>0.13 ± 0.49</td>
</tr>
<tr>
<td>5 years</td>
<td>0.64 ± 2.12</td>
<td>0.51 ± 1.23</td>
<td>1.17 ± 3.19</td>
<td>0.18 ± 1.14</td>
<td>0.13 ± 0.49</td>
</tr>
<tr>
<td>6 years</td>
<td>0.67 ± 1.91</td>
<td>0.48 ± 1.04</td>
<td>1.16 ± 2.82</td>
<td>0.19 ± 0.85</td>
<td>0.12 ± 0.48</td>
</tr>
<tr>
<td>8 years</td>
<td>0.73 ± 1.55</td>
<td>0.48 ± 0.88</td>
<td>1.35 ± 2.47</td>
<td>0.15 ± 0.55</td>
<td>0.16 ± 0.44</td>
</tr>
<tr>
<td>10 years</td>
<td>0.71 ± 1.37</td>
<td>0.41 ± 0.83</td>
<td>1.14 ± 2.36</td>
<td>0.12 ± 0.48</td>
<td>0.16 ± 0.44</td>
</tr>
<tr>
<td>12 years</td>
<td>0.68 ± 1.40</td>
<td>0.43 ± 0.85</td>
<td>1.25 ± 2.31</td>
<td>0.14 ± 0.44</td>
<td>0.16 ± 0.44</td>
</tr>
<tr>
<td>14 years</td>
<td>0.66 ± 1.40</td>
<td>0.40 ± 0.78</td>
<td>1.21 ± 2.31</td>
<td>0.14 ± 0.44</td>
<td>0.16 ± 0.44</td>
</tr>
<tr>
<td>16 years</td>
<td>0.66 ± 1.38</td>
<td>0.37 ± 0.75</td>
<td>1.20 ± 2.26</td>
<td>0.20 ± 0.40</td>
<td>0.15 ± 0.39</td>
</tr>
<tr>
<td>Adults</td>
<td>0.70 ± 1.10</td>
<td>0.50 ± 0.90</td>
<td>1.10 ± 1.70</td>
<td>0.70 ± 1.10</td>
<td>0.20 ± 0.40</td>
</tr>
</tbody>
</table>
Identification of References and Raw Data
We could identify 17 original publications describing lymphocyte subpopulations in children by flow cytometry. Most of these papers were found in citation databases, additional ones were identified by literature searches and in textbooks. In order to improve the database of normal values, we added further data out of our own laboratory.

We focused on the following lymphocyte populations:
- T cells (CD3+),
- T helper cells (CD3+ CD4+ CD8–),
- cytotoxic T cells (CD3+ CD4– CD8+),
- B cells (CD19+),
- NK cells (CD16+ CD56 + CD3–).

Although a number of other subpopulations were described in several of these publications, we decided not to consider rarely reported findings.

In 8 publications the data were reported as mean and standard deviation (SD). Such data were entered into the calculation of age-dependent normal values. This was not feasible for the other 9 papers that published data as median and percentiles, unless the raw data could be obtained from the authors.

Data Collection
Data were collected in tables and investigated by data analysis and comparison methods. In particular, the following data were taken into consideration:
- age, gender,
- sample collection, anticoagulation, pre-analytics, time to workbench,
- definition of normal group, ethnics,
- applied method for immunophenotyping, direct or indirect staining, monoclonal antibodies used,
- cell separation or whole blood protocols,
- instrumentation and software products,
- definition of cell populations, data analysis.

Statistic Analysis and Visual Presentation
Data analysis, statistical examination as well as definition of age-related values are based on calculation of patients’ groups covering relevant age ranges. As a consequence of this approach, the patient’s counts was initially reduced. However, it opened the opportunity to merge it with values from other studies that did not work with equal time spans. Thereby, mean values and standard deviations could be recalculated for these combined data. Non-parametric data were analyzed for fundamentally fitting values. It is noteworthy that there were no two studies that grouped the children into age groups of identical range.

Results
By help of the identified data we could generate a data set describing normal values in short time periods during childhood. Nearly all available publications could be included, except for selected ones. Data generated by the following methods were not included:
- analysis by cell sorters (such data did not fit with the other publications using flow cytometer analyzers)
- analysis by microscopic counting, and
- rosetting techniques instead of monoclonal antibodies.

All other data could be included into our study. We did not find any influence of the following variables on the results:
- gender,
- ethnicity,
- anticoagulant used,
- preparation of sample (ficoll vs. whole blood technology),
- monoclonal antibodies used,
- selected staining protocols,
- technical platform.

The results of the 16 included papers were statistically homogeneous. Cell counts are presented in the following figures. Table 1 shows the mean ± 1 SD range for all investigated cell populations.

It was common for all lymphocyte subsets that there is a substantial dynamic change during the 1st year of life. This finding is missing from previous publications because they are based on too long age ranges. Especially around the 4th to 6th month of life artificial T lymphocytosis could cause misinterpretation.

T-cell count during the 1st year of life was evidently higher than at older age. Especially during the second half of the 1st...
year of life, the number of T cells was higher than expected (fig. 1). Based on most accessible papers, there is a general consensus about this intermediate peak. All published data fit well with our own findings, but the common practice of classification of children into one ‘1st year group’ sometimes obscures this fact and thereby causes underestimation of expected values.

T helper cell counts exhibited an intermediate peak around the 6th month of life, similar to that in T cells (fig. 2). All publications provided homogeneous findings, with a tendency to calculate too small T helper cell counts around the 6th month based on a long time period grouping of the enrolled children. Cytotoxic T cell counts showed – similar to T helper cells – an intermediate peak during the 1st year that is slightly delayed (fig. 3). This result was also consistently found in all publications included.

B cells had smaller counts than T cells, but the kinetics was similar (fig. 4). This is consistently found in nearly all included publications.

The time course of NK cell counts obviously differed from that of the other lymphocytes. From birth on, there is a decreasing number of NK cells in the peripheral blood (fig. 5). However, this tendency could not be recognized in all publications. Frequently, NK cells are reported to have smaller counts.

Discussion

Cytometric analysis is an ideal tool for immunophenotyping, especially in children, for a multitude of reasons. It is a high-throughput technology that allows the rapid and accurate quantitation of even minute cell subsets such as stem or progenitor cells [53] in a few seconds to minutes by measuring tens of thousands to millions of cells. By measuring multiple colors it is easily feasible to detect simultaneously different epitopes on the same cell and thereby enabling its precise phenotyping.

Present technologies allow already to measure 6 colors for routine immunophenotyping and future developments may push this even forward to 17 or more colors [54, 55] by applying sophisticated technology and novel fluorescent colors derived from nanotechnology [8, 56]. There are two additional effects of multiplexed cell analysis:

- The required blood volume for diagnosis is drastically reduced. This is important for children, in particular in critically ill neonates. Progress in instrument and computer development [57] in the future will enable to even more reduce the required sample volume by performing cytometric analysis on the slide in specially designed microscopes [58, 59] that may be substantially cheaper than present instruments [60].

- The complex data pattern that results from the multiplexed measurements will allow to perform computer-aided systemic analysis [61] that may lead to improved risk assessment and hopefully to individualized medicine [8, 62]. Nevertheless, all these multiparametric investigations require reliable normal values that represent inconspicuous findings.
in children without diseases. Our data show that meanwhile flow cytometric data are to a high degree independent of the technological platforms, selected antibodies, or laboratory protocols, when sticking consequently to validated protocols for clinical laboratory diagnostics. Furthermore, this allows merging data and findings out of several studies into one data set as a base for decision making. The high stability must not be true under all conditions. In fact, in immunodeficiencies most detection systems (labeling, instrument, analysis) must be validated under conditions representing realistic symptoms of such diseases. But in healthy persons this aspect is less critical. Time courses of the investigated cell populations underline the necessity to compare flow cytometric diagnostic findings strictly with age-related normal values. Without consideration of changes in early childhood, misinterpretation and misleading diagnostic reports may be generated.

Conclusion

Development-related alterations of lymphocyte subset counts in children could be extracted from the pre-existing papers for diagnostic use. These results were mostly independent of gender, ethnic factors, procedure of sample collection, anticogulation, pre-analytical procedures, time to workbench, applied method for immunophenotyping, staining procedure, selected monoclonal antibodies, technical devices, and software products.

Our data indicate that previous normal values are not sufficiently precise for the interpretation of lymphocyte subsets in children. Mainly during the 1st year of life, count and subset distribution of lymphocytes is different from that of adults. Therefore, a close meshed data set for normal values is required to guarantee adequate diagnostic interpretation.

References

12. Sack Gerl/Ing Tärnok

Downloaded by: 54.70.40.11 - 10/5/2017 8:30:00 AM
Age-Related Lymphocyte Subset Changes in the Peripheral Blood of Healthy Children

Transfus Med Hemother 2007;34:176–181