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Lymphocyte reference intervals in the era of newborn screening (NBS)

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Conflict of Interest

The authors have no conflict of interest to declare

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The need for age specific reference intervals to interpret immunologic laboratory data from infants and children is well accepted and has driven numerous publications providing these data sets for a host of different immunologic tests. This requirement also includes lymphocyte subsets determined by flow cytometry (immunophenotyping) as it represents a commonly used test for evaluating the immune status of pediatric patients. Over the past 25+ years a number of peer reviewed articles describing reference ranges for immunophenotyping in different pediatric age groups have been published (1-6). In addition, it has been documented that the T and B cell compartments undergo critical maturation during fetal development with increasing diversity and complexity of each lymphoid population evolving over 12-26 weeks of gestational age (7). The application of newborn screening (NBS) using the T cell receptor excision circle (TREC) assay in all states within the United States has increased the need for accurate reference intervals for T cell subsets, including naïve T cells, in newborns of varying gestational ages. This arises from the routine use of T cell immunophenotyping as a confirmatory test following an abnormal TREC NBS in order to substantiate or rule out the presence of a T cell immune defect.

In this issue of the Journal of Allergy and Clinical Immunology, Amatuni, et al., provide the most complete data defining the reference ranges for T cell subsets linked to estimated gestational age (EGA), ranging from very premature infants of 22-28 weeks to full term newborns (8). In addition, the authors provide T cell subset reference ranges according to birth weight, as alternative data sets in situations where there are questions regarding EGA. The information herein represents 6.5 years of NBS experience in the state of California, that involved >3.2 million TREC assays. The follow-up approach in the most populous state of the Union involved obtaining a standard multicolor flow cytometric panel performed by a single commercial laboratory. The absolute lymphocyte count using an automated hematology instrument was obtained on the same sample in order to determine the absolute cell number of each lymphocyte subset. All data are presented as the median together with the 5th and 95th percentile (90% confidence interval). In the course of evaluating abnormal TREC results from this large number of NBS, 338 newborns were identified with abnormal TREC results who were determined not to have an immune disorder or other disqualifying medical conditions. From this group of newborns, ranging in gestational age from 22 weeks to term, these investigators were able to generate seven different EGA groupings with sufficient samples (range 26-75 per group) to define the reference ranges. Interestingly, when the data was further analyzed, no significant impact of race/ethnicity (i.e., African American, Asian, Caucasian or Hispanic) was detected on the lymphocyte subsets values. This is not a trivial finding as it might suggest that factors other than race and genetic background (environmental, epigenetic) could have an influence on lymphocyte subsets at early ages.

The selection process for the subjects in this study is somewhat unusual since the triggering point in each case was an abnormal TREC NBS result. This prompted

immunophenotyping of the patient, that ultimately ruled out severe combined immunodeficiency (SCID) or other causes of severe lymphopenia. The authors went to great lengths to prove that all the newborns included in this study did not have an immune defect or other medical condition associated with an expected impact on the development or numbers of T cells. The validity of this data is further supported by the comparability of their full-term newborn data with previous smaller studies that examined lymphocyte subsets in either newborn blood samples or cord blood (Table) (1,2,4). Even though the monoclonal antibody combinations varied in these studies, the absolute lymphocyte and lymphocyte subset values were quite consistent; however, they lacked evaluation of pre-term newborns. Thus, the unique and valuable contribution of this study is related to the reference intervals for lymphocyte subsets from premature (earlier EGA) newborns, data that was not available prior to this publication. These data (Table III) together with the reference intervals connected to varying birth weights (Table IV) from the very premature to the full-term newborn will “help neonatologists and immunologists to provide optimal care, both by recognizing and protecting infants with immunodeficiency and by avoiding excessive anxiety and immune testing when not necessary.” (8)

In addition to this very important clinical application, the data provided in this article forms an valuable reference point for future studies in other countries utilizing TREC NBS. The impetus for determining regional reference intervals is related to the observation that immunophenotyping pediatric subjects in different countries can result in varied reference intervals (9,10). Overall, this article adds novel and clinically useful information on lymphocyte subset reference intervals in newborns of varying EGA, birth weight and ethnic background.

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Table I

	<u>Amatuni, et al</u>	<u>Reference 2</u>	<u>Reference 4</u>
	40-41 wks EGA (n=75)	Neonates (n=20)	Cord blood (n=125)
Lymphocytes	4500 (2600-7770)	4800 (700-7300)	5400 (3100-9400)
CD3	2968 (1512-6092)	2800 (600-5000)	3100 (1400-6800)
CD3/CD4	1980 (912-4725)	1900 (400-3500)	2200 (1000-4800)
CD4 naive	1677 (571-4169)*	Not determined	1800 (900-3900)**

Results given as median (5th/95th) percentile cells/mm³

*CD3+/CD4+/CD45RA+

**CD3+/CD4+/CD45RA+/CD27+