Nele Klose
Dr. med.

Evaluating Subpopulations of Lymphocytes of Adults in Nouna, Burkina Faso: Reference Values and Comparison of Two Methods of Flow Cytometry

Promotionsfach: Infektiologie
Doktorvater: Prof. Dr. med. Hans-Georg Kräusslich

Flow cytometry is the first choice for enumerating lymphocyte subpopulations, and still advances into innovative fields with characterising subsets of T cell differentiation phenotypes. This further increases its importance in monitoring progression of HIV/AIDS. Yet, widespread use is hindered by factors like its costs, different protocols and absence of local reference values.
We therefore compared a cheaper three-colour lyse-and-wash dual platform (DP) Single Tube technique to the established lyse-no-wash single platform (SP) method of MultiSET (MS). The latter requires five test tubes with TruCount beads, rendering it is too expensive for most laboratories in developing countries. In addition, we aimed to establish reference values, including the T cell differentiation phenotypes, by both methods for the research area of Nouna, Burkina Faso.
The simplified Single Tube technique failed the quality control criteria more frequently than MS. In addition, Bland-Altman analysis (bias of the means ±1.96*SD) documented a significant underestimation of the percentages of NK (-2.32 ±6.31) and B cells (-4.54 ±4.82), in favour of CD3+ CD8+ (2.91 ±5.56) and combined CD3+ T cells (4.23 ±5.11). Overall, the haematology analyser (Sysmex KX-21N) caused the most relevant distortions, displaying much lower lymphocyte counts than the SP technique (-473 ±638, p= 0.0004), affecting all compartments.
Still, for longitudinal monitoring of HIV-positive patients in developing countries, the cheaper DP Single Tube appears acceptable for a coarse overview, especially as CD4+ percentage values corresponded between both methods (-0.09 ±7.92, p= 0.9324).

Lymphocyte reference values based on SP MS measurements of 186 healthy adults (89 women, 97 men) between 18 and 78 years (median 25 years) in the study area were considerably higher than reported for most regions outside of Western Africa. The median percentages (absolute counts) for CD3+ cells were 41% CD4+ T cells (1,082/µl) and 24% CD8+ T cells (600/µl), with a median CD4+/CD8+ ratio of 1.7. 70% of all lymphocytes were CD3+ T cells (1,801/µl), 13% CD19+ B cells (336/µl) and 14% CD16+ CD56+ NK cells (352/µl).
Women differed significantly from men in terms of CD3+ percentages, as well as in percentages and absolute counts of CD3+ CD4+ and NK cells.

The differentiation of maturation phenotypes, via CCR7 and CD45RA, included 102 women and 74 men (155 data sets for absolute counts). Considerable variation blurred any potential gender or seasonal difference. Yet overall, decreased proportions of naive cells confirm considerable immune activation in Africa, as compared to Western countries. The median percentage (absolute count in cells/µl) was 21% (223/µl) among CD4+ and 26% (157/µl) among $CD8^{high}$ lymphocytes. Also the CD4+ EMRA population, associated with chronic immune activation by HIV, was elevated in the population studied with 4% (42/µl). The higher proportion of these cells in the $CD8^{high}$ subset, with 33% (221/µl), was consistent with Western publications. The often prevailing central memory cells among CD4 positives constituted only the second largest group with 28% (315/µl). Among CD8 positives, they were by far the smallest subset, with only 2% (15/µl). Instead, the effector memory type dominated both the CD4+, with 44% (471/µl), and the CD8+ compartment, with 35% (223/µl).
Open questions exist regarding reliability of flow-cytometric differentiation of T-cell maturation phenotypes, and application of the Single Tube lymphocyte differential to patients with diseases

such as HIV/AIDS. The impact of methodological and external influences on these parameters, which is highlighted by each study section, may account for some of the reported regional differences between populations all over the world. The fact that such differences are potentially overestimated by the SP MS method also requires further investigation. It also emphasises the need for comparative measurements and reference values that are specific for a given area and particular method, before a new technology can be applied to patients.