Short Communication

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Diurnal variation of leukocyte counts affects the indirect estimation of reference intervals

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Abstract: It has been observed that the estimation of reference intervals of leukocytes in whole venous blood leads to higher upper reference limits (uRLs) with indirect methods than has been reported in the literature determined by direct approaches. This phenomenon was reinvestigated with a newer, more advanced indirect method, and could be confirmed. Furthermore, a diurnal variation was observed with lower values during the morning and higher values in the late afternoon and at night. This observation can explain why indirect approaches using samples collected during 24 h lead to higher uRLs than direct methods applied on samples collected presumably in the morning.

Keywords: diurnal variation; indirect reference intervals; leukocyte reference intervals.

Naus et al. [1] were the first authors who notified that the leukocyte count in whole venous blood estimated by an indirect method led to higher upper reference limits (uRLs) than those reported in the literature determined by direct approaches. The authors found an uRLs of 12.3·10^9/L in a mixed population (45% hospitalised patients, 45% out-patients, 10% blood donors) and cited two references from the literature with 10.0·10^9/L. A more recent study with apparently "healthy" hospital employees reported 3.9–10.9·10^9/L for men and 4.49–12.68·10^9/L for women [2].

Naus et al. [1] stated: “for leukocytes, the frequency distribution could be very well described by two overlapping Gaussian curves. It is quite not clear whether this second curve represents a true subpopulation. The fact, however, that about 30% of all data is part of the second distribution, indicates that they cannot be classified as ‘abnormals’. Possibly, this group represented persons who suffered from mild infections in the recent past”. The two Gaussian distributions were hypothesised and not supported by realistic data.

We reinvestigated the observation of Naus et al. with different subpopulations of one primary health care laboratory and two tertiary health care laboratories with a newer, more advanced indirect approach.

No relevant differences were found between men and women, and no age dependency was observed between 18 and 100 years (Figure 1). Therefore, RIs were estimated for the age interval 18–100 years for men and women combined (Table 1). The reference intervals (RIs) determined during the whole day (0–24 h) were similar to those reported by Naus et al. (3.8–12.31·10^9/L). The RIs of ambulant patients were slightly lower than those of hospitalised patients. The primary care laboratory had the lowest values which were almost identical to the RIs reported by a recent study with a direct method using the same analytical platform [2].

Furthermore, the data sets could be stratified according to the time when the samples arrived in the laboratory. It may be assumed that the transport times are approximately the same for all samples (about 2 h). The daytime variation has a similar pattern from Monday to Friday. If the values from Monday to Friday are combined (Figures 2-4, not stratified by sex and age), a maximum was detected during the night and a minimum in the morning (8:00–12:00). Therefore, uRLs were lower during the morning and higher in the late afternoon (Table 1). A diurnal variation has also been reported by others [5, 6].

The daytime variation can explain why the upper Rls estimated by indirect methods are slightly higher than those derived by direct methods. Whereas indirect methods use samples collected during 24 h, direct methods usually apply samples taken during the morning (close to the nadir) from young, “healthy” subjects, often employed by hospitals [2]. Upper Rls determined in primary health
Laboratory performed as recently described [1] by the Guidelines of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (RiliBAEK) [4]. The TMC method (truncated minimum chi-square) was performed as recently described [5]. RL, reference limits; CL, confidence limits.

Table 1: Reference limits (RLs) with confidence limits (CLs) of leukocyte counts (×10^9/L) in whole blood estimated by the truncated minimum chi-square (TMC) approach. The data sets (n, number of data) were of ambulant and hospitalised women and men (18–100 years). Leukocyte counts were determined in two university hospitals (laboratory 1, university of Kiel, Sysmex XN 1000) and (laboratory 2, university of Bochum, Sysmex XN 1000), and in one laboratory of the primary health care section (laboratory 3, Karlsruhe, Sysmex XE2100). The laboratory results of patients were selected as described in ref. [3]. Laboratory 3 received less than 5% of samples from hospitalized patients. The laboratories which provided the measurement values were accredited according to ISO 15189 and followed the Guidelines of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (RiliBAEK) [4]. The TMC method (truncated minimum chi-square) was performed as recently described [5]. RL, reference limits; CL, confidence limits.

| Day time       | Lower RL | CLs     | Upper RL | CLs     | n     | uPrev^\text{a} |
|----------------|----------|---------|----------|---------|-------|---------------|
| Laboratory 1   |          |         |          |         |       |               |
| 0–24:00        | Ambulant | 3.91    | 3.904–3.923 | 13.39   | 13.357–13.422 | 553,249 | 2.4          |
| 0–06:00        |          | 4.72    | 4.635–4.262 | 16.52   | 16.278–16.628 | 16,808  | 0.7          |
| 10:00–11:00    |          | 3.63    | 3.603–3.658 | 12.34   | 12.202–12.367 | 68,579  | 2.7          |
| 0–24:00        | Hospitalised | 3.91 | 3.904–3.922 | 13.37   | 13.343–13.408 | 995,453 | 2.4          |
| 0–06:00        |          | 4.72    | 4.656–4.721 | 16.52   | 16.154–16.628 | 48,851  | 0.7          |
| 10:00–11:00    |          | 3.63    | 3.603–3.652 | 12.34   | 12.202–12.367 | 136,284 | 2.7          |
| Laboratory 2   |          |         |          |         |       |               |
| 0–24:00        | Ambulant | 4.15    | 4.114–4.184 | 13.28   | 13.167–13.394 | 39,256  | 3.6          |
| 0–06:00        |          | 4.94    | 4.741–5.143 | 15.48   | 14.848–16.110 | 1,807   | 4.2          |
| 8:00–10:00     |          | 3.89    | 3.774–4.012 | 12.02   | 11.657–11.942 | 3,477   | 5.6          |
| 0–24:00        | Hospitalised | 4.23 | 4.204–4.254 | 13.92   | 13.840–14.004 | 100,076 | 8.5          |
| 0–06:00        |          | 4.47    | 4.324–4.615 | 18.54   | 17.944–19.152 | 4,993   | 5.2          |
| 8:00–10:00     |          | 3.77    | 3.681–3.850 | 13.58   | 13.277–13.888 | 8,366   | 4.9          |
| Laboratory 3   |          |         |          |         |       |               |
| Ambulant       |          | 3.94    | 3.918–3.946 | 11.2    | 11.179–11.220 | 862,193 | 5.1          |

^aUpper prevalence in % of the total number of data in the particular subpopulation.
The diagnostic efficiency of leukocytosis could not be studied from the available data sets. However, the prevalence of leukocytosis could be calculated by the truncated minimum chi-square (TMC) procedure as recently described [3]. The prevalences of leukocytosis varied between 0.7 and 2.7% in laboratory 1, and between 3.6 and 8.5% in laboratory 2 (Table 1) and were not higher during the night than during the morning. The prevalences were based on RLs derived from the particular population subsets and were used as a surrogate for the diagnostic efficiency. If the upper RLs seen during the night would be caused by a higher rate of subclinical diseases, the upper prevalence should be higher during the night. This was not the case.

Diurnal variations of lymphocytes in whole venous blood show similar pattern as observed for leukocytes [6, 7]. It is interesting to note that haemoglobin concentrations and erythrocyte counts have an opposite pattern with higher values in the morning and lower values in the afternoon [6, 8].

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**Competing interests:** Authors state no conflict of interest.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Ethical approval:** Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors’ Institutional Review Board or equivalent committee.

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**Figure 3:** Diurnal variation of the leukocyte count in whole venous blood (hospitalised patients, laboratory 2, n=100,076). Daily observations are calculated during one week and the medians from Monday to Friday are presented (×10⁹/L). The yellow areas represent the approximate 95% confidence limits based on the median absolute deviation.

**Figure 4:** Diurnal variation of the leukocyte count in whole venous blood (ambulant patients, laboratory 2, n=39,256). Daily observations are calculated during one week and the medians from Monday to Friday are presented (×10⁹/L). The yellow areas represent the approximate 95% confidence limits based on the median absolute deviation.

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