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**Effect of age and gender on reference intervals of red blood cell distribution width (RDW) and mean red cell volume (MCV)**

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**Abstract**

**Background:** Red blood cell distribution width (RDW) was recently shown to be age-dependent when using Sysmex XE-2100 hematology analyzers. As measuring RDW is subject to technology, we have investigated if this relation also exists when using a different hematology analyzer, Abbott CELL-DYN Sapphire. In addition, as RDW is generally expressed relative to mean red blood cell volume (MCV), we have explored how MCV influences the age-dependency of RDW.

**Methods:** We measured RDW and MCV in a large cohort and calculated RDW-SD (the “absolute” RDW), which does not contain MCV. For establishing reference intervals we used Bhattacharya statistics.

**Results:** In our study cohort of 8089 individuals we found a strong association between RDW and age, but not with gender. Also MCV showed an age-related increase over the entire age range. The conventional RDW increased by 6% from the youngest to oldest age class, whereas RDW-SD increased by nearly 15%. This difference was caused by a mean age-related increase in MCV of 6.6%. Age-dependent reference intervals were established for RDW, RDW-SD and MCV.

**Conclusions:** The age-dependency of RDW seems to be a universal biological feature rather than related with a single type of hematology analyzer. As not only RDW, but also MCV increases with age, we propose that future studies on the prognostic significance of RDW take its age-dependency into account and focus on RDW-SD as a potential marker of adverse events in many clinical conditions.

**Keywords:** age-dependency; gender-dependency; mean cell volume; red cell distribution width; reference intervals.

**Introduction**

The medical and laboratory literature has recently seen massively growing interest in the red blood cell distribution width (RDW) [1], after Felker’s description of RDW as a prognostic marker in heart failure patients [2]. Also this journal has published various papers on RDW [3, 4] and recently the age- and gender-dependency of RDW was addressed [5, 6]. The conclusion from these two latter studies was that RDW predominantly depends on age, while gender differences were of less significance. As previously pointed out, RDW is a parameter which lacks international standardization, meaning that analyzers from different manufacturers may produce different RDW values in the same blood samples [7, 8]. Indeed it has been demonstrated that considerable inter-instrument differences in RDW results do exist, which automatically implicates the need for adequate, analyzer-specific reference intervals. The two recent reports on age-dependency of RDW values both used the Sysmex XE-2100 analyzer and the results of both studies were quite similar [5, 6]. Therefore the question arises whether these findings can directly be translated to RDW measured with other analyzers.

Another potential issue with RDW is that it is measured as the standard deviation of the RBC volume distribution curve (in fL, the same unit as RBC volume), but practically always is reported as a percentage, relative to mean RBC volume MCV [1, 8]. There are indications that also MCV is age-dependent [9–12], so another dependency might be introduced in the RDW calculation; until present this aspect has not been addressed in the literature. It could thus be of interest to eliminate the influence of MCV from RDW, which is readily possible by calculating the “absolute” RDW as a standard deviation (RDW-SD) of the RBC volume distribution curve, expressed in fL.
The aims of the current study were threefold: first, to explore the age- and gender-dependencies of RDW as measured using an Abbott CELL-DYN Sapphire hematology analyzer. The second aim was to investigate to what extent the MCV age-dependency is of practical significance for the age-dependency of RDW and finally we aimed to establish age- and gender-specific RDW reference intervals for the hematology analyzer mentioned.

Materials and methods

Study design

Our study was originally designed for establishing reference intervals of the new extended RBC and reticulocyte parameters in CELL-DYN Sapphire, as described previously in detail [12]. Obviously, other blood cell parameters were recorded in our database, too, enabling to study RDW and MCV in relation to subject age and gender. During a period of 4 weeks we measured a complete blood count in all subjects for whom hematological investigation was requested in the “Diagnostiek voor U” Primary Care laboratory. We cleaned the database by excluding samples from patients that had blood drawn more than once during the study period [12]; no further selection was performed. As we used residual blood samples that had been collected for diagnostic purposes, the study was exempt from assessment by the Ethical Review Board.

Analytical methods

All blood samples were collected into K$_2$-EDTA evacuated tubes and processed within 6 h using CELL-DYN Sapphire hematology analyzers (Abbott Diagnostics, Santa Clara, CA, USA), following the manufacturer’s guidelines. The laboratory’s standard operating procedures, including internal and external quality control, were in full accordance with the requirements of CCKL, the Dutch Authority for Medical Laboratory Accreditation. We used RDW and MCV values as reported by the analyzer and calculated RDW-SD values using the formula: RDW-SD = RDW (%)×MCV (fL)/100; the unit of RDW-SD expression is fL. The analytical precision of these parameters measured during the study period, expressed as coefficient of variation of a normal control sample, was 0.68% for RDW, 0.93% for RDW-SD and 0.67% for MCV.

Statistical analysis

Normality of the data distributions was checked using the Kolmogorov-Smirnoff test. For investigating age- and gender-dependency we used Kruskal-Wallis analysis of variance with post-hoc analysis according to Conover. Comparison between groups was done using the Mann-Whitney test. Statistical significance was assumed at p<0.05. All statistical tests were performed using MedCalc for Windows software (version 14; MedCalc, Oostende, Belgium) [13].

As our study enrolled unselected subjects, some of whom were likely not healthy, we applied Bhattacharya statistics for calculating the reference intervals of RDW, RDW-SD and MCV in men and women separately, for the various age classes as mean ±2 SD. This technique is capable unveiling the underlying normal distribution [12, 14].

Results

After exclusion of repeat visits we eventually had data from 8089 unique individuals in our database; 5216 were females (median age 51 years, range 1–102 years) and 2873 males (median age 57 years, range 0–106 years). Using current WHO criteria, a total of 1664 subjects had anemia: 1029 women with Hb <12.0 g/dL and 624 men with Hb <13.0 g/dL). These anemic subjects were excluded from the investigation of age- and gender-dependency of RDW and MCV.

Initial statistical analysis revealed that none of the data-sets was normally distributed, also not after logarithmic transformation; therefore we decided to use non-parametric statistical methods. We found a significant correlation between RDW and age in females (Spearman’s rho 0.194, with 95% confidence interval 0.165–0.223; p<0.001) as well as in males (Spearman’s rho 0.201; 95% CI 0.161–0.240; p<0.001). We divided the subjects into convenient age classes. Figure 1A illustrates the age-dependent increase of RDW in these classes in both sexes. There was a significant albeit very small difference in RDW between women and men in the entire cohort (p=0.0214): women median (and interquartile range, IQR in parentheses) 12.03% (IQR 11.68–12.56) and men 12.05% (IQR 11.69–12.55). None of the age classes demonstrated a significant gender difference in RDW, although men in the 56–70 years class showed a tendency to have a somewhat higher RDW (12.10%; IQR 11.72–12.62) than women of the same age (12.06%; IQR 11.67–12.51; p=0.0917 by Mann-Whitney test). Analysis of variance confirmed that RDW was significantly associated with age (p<0.001) in all age classes; the differences between all age classes were significant with one single exception (41–55 years and 56–70 years classes in females).

For the calculated RDW-SD we also found a strong correlation with age (Spearman’s rho 0.301; 95% CI 0.279–0.324; p<0.001), as shown in Figure 1B. Every age class was significantly different from all other classes with respect to RDW-SD (p<0.001). Overall, men appeared to have significantly higher RDW-SD than women (p=0.037 by Kruskal-Wallis test). However, the difference was minimal: median values were 11.02 fL (IQR 10.57–11.60) in males and 10.96 fL (IQR 10.52–11.56) in females, respectively. In subgroup analysis, a significant gender difference was only found in...
the 56–70 years age class (p=0.014 by Mann-Whitney test). However, this difference was minor, too: men had median 11.13 fL (IQR 10.73–11.67) compared to women median 11.03 fL (IQR 10.63–11.59). The other age classes did not exhibit significant gender differences in RDW-SD.

When MCV was investigated, a similar pattern emerged: MCV correlated significantly with age (Spearman’s rho 0.206; 95% CI 0.182–0.229; p<0.001), as shown in Figure 1C. Kruskal-Wallis analysis of variance confirmed the age-dependency in the youngest 4 classes (all p<0.001) and showed no association of MCV with gender (p=0.257).

As RDW calculation conventionally includes MCV, we examined how these two parameters were related. Figure 2A demonstrates that RDW decreased significantly with increasing MCV in the first four quintiles (all p<0.001 by Kruskal-Wallis test), and then slightly, but insignificantly increased in the highest MCV quintile. There was no influence of gender. In contrast, the absolute RDW (RDW-SD) showed a highly significant positive correlation with MCV (Spearman’s rho 0.536; IQR 0.519–0.553; p<0.001). Analysis of variance confirmed that RDW-SD was significantly associated with MCV (all classes p<0.001), irrespective of gender, as illustrated by Figure 2B.

Finally, reference intervals for RDW were calculated for both genders using the Bhattacharya method; the results are shown in Table 1. It is evident that RDW demonstrates an age-dependent increase in reference intervals, irrespective of how RDW is expressed (RDW or RDW-SD). Table 2
show the reference intervals of MCV for the same age classes; age-dependency is obvious, but relatively small.

**Discussion**

Our findings regarding RDW and age corroborate those reported by Lippi et al. [5] and Alis et al. [6], but in contrast to their findings we were unable to confirm that gender plays a relevant role as a determinant of RDW. The reason for this discrepancy might be related to the number of observations. As our study cohort was 4 and 10 times larger than the other studies respectively [5, 6], we would have expected at least a trend in significance if a gender effect were present in our study. Therefore we believe that if gender plays a role in RDW, it is of no relevance and does not require gender-specific reference intervals (Table 1).

It is well documented that the measuring technology and algorithmic approaches used by hematology analyzers can lead to differences in RDW [7, 8]. This does not only result in different reference intervals, but we have also shown that the width of reference intervals varies between different analyzers [7]. In the present study we have used CELL-DYN Sapphire hematology analyzers and found that the age-dependency of RDW was very similar to previous data that were generated using Sysmex XE-2100 instruments [5, 6]. Our results thus suggest that the make of the hematology analyzer and details of mathematic algorithms are no relevant factors for the relationship between RDW and age. It will be of interest to see whether future studies using other hematology analyzers will confirm the age-dependency of RDW as an analyzer-independent, universal biological finding.

Other researchers have reported an age-related increasing proportion of subjects with RDW values >14.6%, which was considered as the conventional upper limit of reference interval [5, 6]. We find this approach disputable, as it does not take age-related RDW reference intervals into account. Moreover, applying a fixed clinical decision value should anyway be discouraged as RDW reference intervals are analyzer-dependent [7, 9]. There is an obvious need for clinical studies on the prognostic value of RDW that incorporate the age-dependency of RDW into their design.

When comparing the effects of age, the normalized difference between the lowest and highest age classes is considerably larger for RDW-SD than for the traditional RDW, namely nearly 15% and 6%, respectively (Tables 1 and 2). This is the consequence of the fact that also MCV increases with age, by approximately 6.6% across the

**Table 1:** Reference intervals of RDW and RDW-SD for both genders, calculated using Bhattacharya statistics.

<table>
<thead>
<tr>
<th>Age class, years (females/males)</th>
<th>n</th>
<th>RDW females, %</th>
<th>MCV females, fl</th>
<th>RDW males, %</th>
<th>MCV males, fl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean±SD</td>
<td>RI</td>
<td>Mean±SD</td>
<td>RI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RI</td>
<td></td>
<td>RI</td>
<td></td>
</tr>
<tr>
<td>&lt;18  (females/males)</td>
<td>236 (139/97)</td>
<td>11.9±0.6</td>
<td>10.7–13.0</td>
<td>12.3±0.6</td>
<td>11.2–13.5</td>
</tr>
<tr>
<td>18–40 (females/males)</td>
<td>2423 (1852/571)</td>
<td>12.3±0.7</td>
<td>11.0–13.7</td>
<td>12.1±0.5</td>
<td>11.0–13.1</td>
</tr>
<tr>
<td>41–55 (females/males)</td>
<td>1565 (925/640)</td>
<td>12.1±0.5</td>
<td>11.1–13.1</td>
<td>12.2±0.6</td>
<td>11.1–13.3</td>
</tr>
<tr>
<td>56–70 (females/males)</td>
<td>1666 (902/764)</td>
<td>12.3±0.6</td>
<td>11.2–13.4</td>
<td>12.3±0.6</td>
<td>11.1–13.5</td>
</tr>
<tr>
<td>71–85 (females/males)</td>
<td>1479 (914/565)</td>
<td>12.6±0.8</td>
<td>11.0–14.1</td>
<td>12.6±0.8</td>
<td>11.1–14.1</td>
</tr>
<tr>
<td>&gt;85 (females/males)</td>
<td>373 (274/99)</td>
<td>12.7±0.7</td>
<td>11.3–14.1</td>
<td>12.9±0.8</td>
<td>11.4–14.4</td>
</tr>
</tbody>
</table>

**Table 2:** Reference intervals of MCV for both genders, calculated using Bhattacharya statistics.

<table>
<thead>
<tr>
<th>Age class, years (females/males)</th>
<th>n</th>
<th>MCV females, fl</th>
<th>MCV males, fl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RI</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RI</td>
<td></td>
</tr>
<tr>
<td>&lt;18  (females/males)</td>
<td>236 (139/97)</td>
<td>87.3±4.2</td>
<td>78.8–95.8</td>
</tr>
<tr>
<td>18–40 (females/males)</td>
<td>2423 (1852/571)</td>
<td>89.8±4.5</td>
<td>80.7–98.9</td>
</tr>
<tr>
<td>41–55 (females/males)</td>
<td>1565 (925/640)</td>
<td>90.0±5.1</td>
<td>80.7–101.0</td>
</tr>
<tr>
<td>56–70 (females/males)</td>
<td>1666 (902/764)</td>
<td>91.1±4.4</td>
<td>83.1–100.8</td>
</tr>
<tr>
<td>71–85 (females/males)</td>
<td>1479 (914/565)</td>
<td>91.7±4.6</td>
<td>82.4–100.9</td>
</tr>
<tr>
<td>&gt;85 (females/males)</td>
<td>373 (274/99)</td>
<td>91.6±4.5</td>
<td>82.5–100.7</td>
</tr>
</tbody>
</table>

RI, reference interval.
entire age range. Therefore, expressing RDW as a percentage of MCV attenuates the influence of age. Or in other words, by expressing RDW relative to MCV, information is lost and this might limit the clinical relevance of RDW in a huge variety of diseases [1]. Thus it is worthwhile studying the prognostic value of RDW-SD as a biomarker of morbidity and mortality and see whether it is a stronger predictor of adverse events than conventional RDW. Notably, we also found that the increase in RDW-SD is steeper for men than for women (Figure 2B) and it remains to be seen if this will translate into gender differences in the prognostic value of RDW-SD.

In conclusion we have demonstrated that the age-dependency of RDW is not only observed when Sysmex analyzers are used, but is also true for the Abbott CELL-DYN Sapphire hematology analyzer. In contrast to other investigators, we could not establish a significant gender effect on RDW. Therefore we have presented only age-dependent RDW reference intervals for this analyzer. Our study also confirmed that MCV increases with increasing age in the general population and as a novel finding we have demonstrated that due to this increase, the age effect on the traditional RDW is attenuated. Therefore we propose that future studies on the prognostic value of RDW will focus on RDW-SD (the “absolute” RDW), which does not include MCV in the calculation. Furthermore, the age-dependency of RDW should be taken into account when designing studies on the prognostic utility of RDW (or RDW-SD) as a marker of adverse events in a multitude of clinical conditions.

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References