TC-related markers are early and responsive indicators of changes in vitamin B_{12} status. Some indication of the size of what constitutes a significant change in serial holo-TC values is provided by the calculation of RCVs. The present study indicates that holo-TC values would need to change by nearly 50% for an investigator to be 95% certain that one value was significantly different from a previous value.

Regression dilution describes the attenuation in a regression coefficient when a single measured value of a covariate is used instead of the usual or mean value over a period of time. The simple method of adjusting regression coefficients for this dilution arises out of measurement error theory and is easily implemented (15). In the case of a single covariate (simple linear regression), correction for regression dilution is achieved by multiplying the regression coefficient by a correction factor. The correction factor is simply the inverse of the RC. For holo-TC, it is 1.26, comparable to a value of 1.14 obtained for homocysteine (16). The adjusted regression coefficient is, therefore, larger than the naive coefficient; cross-sectional studies that use regression analysis based on a single assay of holo-TC may therefore underestimate the magnitude of any risk associations with disease by \( \sim 26\% \) in an elderly population.

Our results confirm that, in an elderly population and under the conditions used, this particular holo-TC assay has a desirable quality specification, with analytical variation being approximately one-half that of within-participant biological variation. The results also provide essential information for the interpretation of holo-TC values in both cross-sectional and longitudinal studies in the elderly.

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**References**


**Age- and Sex-related Reference Values for Serum Adhesion Molecule Concentrations in Healthy Individuals: Intercellular Adhesion Molecule-1 and E-, P-, and L-Selectin, Anne Ponthieux, Bernard Herbetter, Suzanne Droesch, Daniel Lambert, and Sophie Visvikis** [Institut National de la Santé et de la Recherche Médicale (INSERM) U 525, 30 rue Lionnois, 54000 Nancy, France, and Centre de Médecine Préventive, 2 rue du Doyen Jacques Parisot, 54500 Vandoeuvre-lès-Nancy, France; * address correspondence to this author at: INSERM Unité 525, 30 rue Lionnois, 54000 Nancy, France; fax 33-03-83-32-13-22, e-mail Sophie.visvikis@cmp.u-nancy.fr]

Intercellular adhesion molecule-1 (ICAM-1) and E\( \rightarrow \), P\( \rightarrow \), and L-selectin are cellular adhesion molecules involved in the recruitment of leucocytes on the activated vessel wall during inflammation (1) and play an important role in the early stages of atherosclerosis and its complications (2). Thus, the measurement of soluble adhesion molecules in serum may have diagnostic relevance in many inflammatory diseases (3). A profile of soluble adhesion molecule concentrations may allow better therapeutic decisions in inflammatory and autoimmune disorders, infection, cancer, and cardiovascular pathologies and may also aid in the prediction of cardiovascular events (4, 5). However, the use of these markers in clinical practice depends critically on knowledge of their reference values.

The purpose of the present study was to establish age- and sex-specific reference intervals for serum concentrations of soluble ICAM-1 and E\( \rightarrow \), P\( \rightarrow \), and L-selectin in healthy children (4–17 years) and adults (18–55 years).

Blood samples were taken from healthy individuals (157 boys and 146 girls 4–17 years of age and 245 men and 250 women 18–55 years of age) who were members of the Stanislas cohort (6). Participants were of French origin (Vosges and Meurthe et Moselle); free from serious and/or chronic illnesses, especially cardiovascular, hepatic, or renal diseases; and were not on treatment with lipid-lowering drugs. Volunteers with aspartate aminotransferase, alanine aminotransferase, or \( \gamma \)-glutamyltransferase activities >200 U/L, apolipoprotein E concentrations >200 mg/L, orormosocid or haptoglobin
concentrations >3 g/L, cholesterol or triglyceride concentrations >10 mmol/L, C-reactive protein concentrations >30 mg/L, or glucose concentrations >8 mmol/L were excluded. The study was approved by the local ethics committee of Nancy (France), and each participant gave written informed consent.

Venous blood samples were collected by venipuncture after an overnight fast (7) and centrifuged (1500g for 15 min at 4 °C) within 2 h after collection. Serum samples obtained were immediately frozen at −196 °C in liquid nitrogen until analysis (storage period, 21–39 weeks; mean, 31 weeks).

ICAM-1 and E-, P-, and L-selectin concentrations were measured with commercially available ELISAs (R&D System). The intra- and interassay CVs for the adhesion molecules were as follows: ICAM-1, 6.6% and 8.6%; E-selectin, 9.4% and 15%; P-selectin, 5.8% and 7.0%, and L-selectin, 8.9% and 12%.

Statistical analyses were performed with SAS software, Ver. 8.01 (SAS Institute Inc.). Because the distribution of serum concentrations of ICAM-1 and E-, P-, and L-selectin were skewed, log_{10} transformations were applied. ANOVA and pairwise comparison procedures with Bonferroni correction were performed to test differences in soluble ICAM-1 and E-, P-, and L-selectin concentrations between age and sex groups. Reference values were estimated using a nonparametric method (5th and 95th percentiles).

Table 1. Mean, median, and age-specific reference intervals based on the 5th and 95th percentiles for ICAM-1 and E-, P-, and L-selectin concentrations in serum.

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1, mg/L</th>
<th>E-Selectin, mg/L</th>
<th>P-Selectin, mg/L</th>
<th>L-Selectin, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Mean (SD) Median RI</td>
<td>Mean (SD) Median RI</td>
<td>Mean (SD) Median RI</td>
<td>Mean (SD) Median RI</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–9 years</td>
<td>12 317 (57) 300 251–437</td>
<td>72.5 (25.6) 70.7</td>
<td>30.5–111.7 150 (48) 147</td>
<td>97–272 1769 (451) 1600</td>
</tr>
<tr>
<td>10–14 years</td>
<td>59 295 (62) 299 208–387</td>
<td>71.1 (32.8) 66.7</td>
<td>31.7–131.5 146 (45) 141</td>
<td>75–232 1419 (495) 1361</td>
</tr>
<tr>
<td>5–17 years</td>
<td>86 268 (63) 261 192–354</td>
<td>62.0 (28.5) 59.4</td>
<td>26.6–102.1 136 (41) 139</td>
<td>67–204 1364 (530) 1235</td>
</tr>
<tr>
<td>18–34 years</td>
<td>70 259 (69) 258 176–363</td>
<td>56.7 (33.3) 47.4</td>
<td>19.1–101.5 147 (39) 144</td>
<td>84–213 1300 (608) 1159</td>
</tr>
<tr>
<td>35–44 years</td>
<td>92 269 (65) 262 181–379</td>
<td>60.3 (26.4) 59.2</td>
<td>22.5–103.1 141 (49) 136</td>
<td>78–215 1086 (455) 1025</td>
</tr>
<tr>
<td>45–55 years</td>
<td>83 262 (64) 263 152–362</td>
<td>54.8 (25.1) 48.7</td>
<td>20.3–105.5 144 (41) 142</td>
<td>84–213 963 (253) 911</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–9 years</td>
<td>11 323 (54) 319 242–410</td>
<td>74.2 (26.5) 72.1</td>
<td>28.5–117.1 140 (37) 138</td>
<td>86–193 1808 (562) 1747</td>
</tr>
<tr>
<td>10–14 years</td>
<td>64 287 (53) 279 221–389</td>
<td>67.5 (31.3) 63.5</td>
<td>23.9–117.0 134 (35) 132</td>
<td>91–183 1410 (477) 1219</td>
</tr>
<tr>
<td>15–17 years</td>
<td>71 258 (55) 261 155–344</td>
<td>49.5 (21.0) 48.0</td>
<td>19.7–84.6 123 (38) 114</td>
<td>61–200 1307 (337) 1288</td>
</tr>
<tr>
<td>18–34 years</td>
<td>73 251 (62) 241 165–374</td>
<td>42.2 (22.6) 41.3</td>
<td>9.3–88.5 116 (33) 117</td>
<td>73–179 1262 (535) 1144</td>
</tr>
<tr>
<td>35–44 years</td>
<td>122 250 (62) 251 152–338</td>
<td>46.1 (24.7) 44.3</td>
<td>10.4–83.0 129 (32) 130</td>
<td>80–181 1082 (466) 985</td>
</tr>
</tbody>
</table>

* RI, reference interval.
immune disorders, inflammation, cancer, and cardiovascular disease (3). Such high concentrations of circulating adhesion molecules may be of interest for diagnostic and prognostic purposes, as demonstrated for ICAM-1 and P-selectin, which provide useful information in healthy individuals and in patients with cardiovascular diseases (9). However, reference values for healthy children and adults have not been defined.

In the current study, we measured serum ICAM-1 and E-, P-, and L-selectin concentrations and determined their age and sex-specific reference intervals over a wide age range (4–55 years) in a healthy population of both sexes.

Earlier studies dealing with serum adhesion molecule (ICAM-1 and E-selectin) concentrations in healthy pediatric populations pointed to an age dependency (10–12). In this study, we observed that mean serum ICAM-1 and E-, P-, and L-selectin concentrations steadily decrease during childhood. ICAM-1 and P-selectin concentrations did not vary with age in adulthood, whereas L-selectin decreased in both sexes and E-selectin increased in women only. Although the decrease in adhesion molecule concentrations with age in children has already been described, its physiologic significance during normal development is unknown. Authors of previous studies in adults observed that ICAM-1, E-selectin, and P-selectin concentrations did not vary with age between 18 and 65 years (3, 5, 8, 10, 13, 14), but the decrease in L-selectin during adulthood has not been described.

ICAM-1, E-selectin, and P-selectin concentrations display a significant sex dependency only in adults, with men having higher concentrations than women. These sex-related differences, as reported previously in healthy adults (8, 14–16), are probably partly attributable to steroid hormones, especially estrogen. Indeed, healthy postmenopausal women on hormone replacement therapy had lower concentrations of these adhesion molecules than controls not on replacement therapy (7, 17, 18), and in vitro studies have shown negative regulation of adhesion molecule expression by estrogen (19).

In conclusion, we have shown that serum ICAM-1 and E-, P-, and L-selectin concentrations are age-dependent in childhood and sex-dependent in adulthood. Application of age- and sex-adjusted reference intervals appears to be necessary. These findings emphasize the need to use age- (for children) and sex-matched controls (for adults) in all analyses of the possible clinical impact of circulating concentrations of adhesion molecules.

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References


Zymographic Analyses and Measurement of Matrix Metalloproteinase-2 and -9 in Nipple Aspirate Fluids, Ferdinando Mannello* and Maurizio Sebastiani† (Istituto di Istologia & Analisi di Laboratorio, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università degli Studi “Carlo Bo”, Via E. Zeppi, 61029 Urbino-PU, Italy; Centro di Senologia, ASL-1, 61100 Pesaro, Italy; * author for correspondence: fax 39-0722-322370, e-mail f.mannello@uniurb.it)

The matrix metalloproteinases (MMPs) are Ca2+/Zn2+ endopeptidases involved in extracellular matrix (ECM) degradation (1) in both tissue remodeling (2) and tumor growth and invasion (3). MMP-2 (gelatinase A; EC