Reference Values and Biological Variation for Tumor Marker CA 19-9 in Serum for Different Lewis and Secretor Genotypes and Evaluation of Secretor and Lewis Genotyping in a Caucasian Population

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The concentration of the tumor marker CA 19-9 is influenced by the patient’s secretor status and Lewis genotype. The aim of this study was to establish novel reference intervals for CA 19-9 in serum based on secretor and Lewis genotypes, to investigate the biological variation of CA 19-9, and to evaluate the utility of Lewis and secretor genotyping on a group of individuals with serologically defined Lewis phenotypes. CA 19-9 was measured in serum of 500 healthy individuals. Secretor and Lewis genotypes were determined by sequencing and PCR-cleavage methods. Significant differences were found between subgroups with different Lewis and secretor genotypes. Genotype-based reference intervals for CA 19-9 are presented. The upper reference limit for all individuals was 28.7 kilounits/L; for secretors and nonsecretors, the upper reference limits were 12.4 and 61.2 kilounits/L, respectively. The analytical imprecision (CVA) was 9.8%, the within-subject variability (CVI) was 15.8%, and the between-subject variability (CVG) was 102.2%. Good agreement was found between Lewis and secretor genotyping and conventional blood grouping. Genotype-based reference intervals may be a way to increase the clinical utility of CA 19-9. On the basis of the calculation of a critical difference for sequential values (significant at \( P < 0.05 \)) of 51.5%, a 40–50% change in marker concentration is suggested as the limit for significant change when the marker is used for follow up. PCR-based genotyping is a reliable method for secretor and Lewis histo-blood grouping.

The Lewis histo-blood group antigens Lewis a (Le\(^a\))5 and Lewis b (Le\(^b\)) are carbohydrate structures that form epitopes on glycolipids and glycoproteins. Two independent genes determine the Lewis phenotype; the Lewis gene (\( Le \) and \( le \)), and the secretor gene (\( Se \) and \( se \); Fig. 1). Combinations of secretor and Lewis genotypes and the resulting erythrocyte phenotypes are shown in Table 1. Conventional Lewis blood grouping is difficult (e.g., in cancer patients and pregnant women) because of the presence of nongenuine Lewis-negative individuals (1). Secretor status in Lewis-negative individuals is determined with a labor-intensive hemagglutination inhibition technique that uses heat-inactivated saliva. In Lewis-positive individuals, secretor status is deduced from the Lewis phenotype: Le(a\(^-\)b\(^+\)) individuals are secretors, and Le(a\(^-\)b\(^-\)) individuals are nonsecretors.

Recently, the Lewis gene (\( FUT3 \)) and the secretor gene (\( FLUT2 \)) were cloned (2, 3), and several silent alleles that cause the Lewis-negative (4–9) and the nonsecretor (10–15) phenotypes, respectively, were identified. Knowledge of the prevalence of the mutated Lewis alleles in a Caucasian population (9) has prompted us to develop a genotyping strategy in which we screen for the presence of...
of two representative mutations, T59G and C314T. An enzyme-inactivating mutation (G428A) in FUT2, which causes a premature stop codon (Trp143→Ter), has been reported to be associated with the nonsecretor phenotype in Caucasian people (12,16), and a straightforward PCR-cleavage assay for the detection of the mutation has been published (13).

The tumor marker, CA 19-9, which recognizes the sialylated Lea carbohydrate structure (17), has been used mainly for serological diagnosis and follow up of gastrointestinal and pancreatic malignancies (18–21). Currently, CA 19-9 is regarded as the most sensitive and specific marker in the diagnosis and follow up of pancreatic cancer. However, the interpretation of CA 19-9 can be difficult because ~7% of the population are Lewis-negative and have undetectable concentrations of CA 19-9 regardless of the tumor burden, and several nonmalignant diseases have been associated with increased serum concentrations of CA 19-9 (18).

The biosynthesis of the sialylated Lea structure is complex and involves at least three genes, FUT3, FUT2, and a sialyltransferase gene, that interact and compete with each other to produce the final products (Fig. 1).

We have shown previously that the secretor status and the Lewis genotype influence the CA 19-9 concentration in serum and in urine (9,22). Because of competition for precursor substrate between the α1-2-fucosyltransferase and the sialyltransferase that initiate the synthesis of the two antigens, sialyl-Lea and Leb, along mutually exclusive pathways (Fig. 1), nonsecretor individuals have more CA 19-9 in their serum and urine than secretor individuals (23). A gene dosage effect may cause Lewis homozygous wild-type individuals (Le/Le) to synthesize more CA 19-9 than individuals who are heterozygous mutated in the Lewis gene (Le/le). On the basis of these investigations, we have hypothesized that the use of genotype-based reference intervals would improve the clinical performance of this tumor marker.

The aim of this study was to establish novel reference intervals for CA 19-9 measurements in serum based on secretor and Lewis genotypes, to investigate the biological variation of CA 19-9 in serum, and to evaluate the utility of Lewis and secretor genotyping on a group of individuals with serologically defined Lewis phenotypes.

Materials and Methods

SUBJECTS AND SPECIMENS

Serum for CA 19-9 measurements and peripheral blood leukocytes for DNA extraction were obtained from 500 apparently healthy volunteers. Of these, 189 participants were recruited among schoolteachers who participated in an environmental study of health complaints caused by indoor climate, and 311 were recruited among military personnel. Participants were not questioned as to state of health. One hundred and eighteen were women, ages 21–69 years, (mean, 46.1 years), and 382 were men, ages 21–66 years (mean, 36.8 years).

For the study of CA 19-9 variability, blood samples from 13 individuals (all women), recruited from among the employees of the hospital, were taken at 1-week intervals, for a total of three times.

All procedures were approved by the local scientific ethics committee.

ERYTHROCYTE PHENOTYPES

Lewis phenotypes and secretor status were determined on K3EDTA-stabilized fresh blood samples by standard agglutination methods in a specialized blood-grouping laboratory at Aarhus University Hospital, Skejby, Denmark. Typing was carried out with monoclonal Lea and Leb antibodies (Seraclone; Biotest). Controls (two positive and one negative) were as follows: for Lea, the controls were O Le(a+b–), A1 Le(a+b–), and B Le(a–b+); for Leb, the controls were O Le(a–b+), A1 Le(a–b+), and A1B Le(a+b–).

LEWIS GENOTYPING

A Puregene DNA Isolation Kit (Gentra Systems) was used according to the manufacturer’s protocol to extract DNA from peripheral blood leukocytes.

PCR-cleavage assays were used to perform Lewis genotyping for the mutations T59G and C314T, as described previously (9). PCR products for T59G and C314T were obtained with the primer pairs VE1 mms/EL3as (5’-
The effect of storage was examined by four repeated measurements of 12 samples (6 high and 6 low concentrations) for a period of 60 days (double determinations). Between analyses, samples were kept at −20 °C. The coefficient of variation (CV) for the high-concentration samples was 8.8%; for low-concentration samples, the CV was 11.0%. No systematic changes in serum CA 19-9 values were observed.

### Statistical analysis

The Solo Statistical System (BMDT Statistical Software) program package was used for statistical analysis. Data were tested for normality with the Martinez-Ingelwicz test. Differences between groups were analyzed non-parametrically by the Mann–Whitney U-test. P <0.05 was considered significant. The kappa measurement of agreement was calculated as described by Armitage and Berry (24).

The analysis of imprecision was performed as described by Fraser and Harris (25). Three CVs were calculated: analytical (CV_A), within-subject (CV_I), and between-subject (CV_G). The CV_A was estimated from replicate analysis of three samples in a single assay. The index of individuality was calculated as CV_I/CV_G, and the “critical difference” (i.e., the percentage difference that is significant at P ≤0.05) was calculated as: 2.77 × (CV_A² + CV_G²)½. Testing for outliers was performed as described by Solberg and Gräsbeck (26). Three points were identified and removed.

### Results

#### Lewis and Secretor genotyping

The results of Lewis genotyping for the mutations T59G and C314T, which are representative of the mutated Lewis alleles present in the Caucasian population, are presented in Table 2. Of the samples tested, 253 (50.6%) were homozygous wild type, 208 (41.6%) were heterozygous mutated for either T59G or C314T, and 39 (7.8%) were mutated in both alleles (T59G homozygous, C314T homozygous or T59G heterozygous, and C314T heterozygous). Allele frequencies were 71.4% for the wild-type allele, 10.3% for the mutated allele T59G, and 18.3% for the mutated allele C314T. The Lewis genotypes were in Hardy-Weinberg equilibrium.

The kappa measurement of agreement between conventional blood grouping of erythrocytes and the PCR genotyping method was calculated as 0.81, which indicates that there is good agreement between the two

### Table 2. Distributions of Lewis genotypes and phenotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T59G Cases (%)</th>
<th>C314T Cases (%)</th>
<th>Erythrocyte phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous wild-type (Le/Le)</td>
<td>253 (50.6)</td>
<td>253 (50.6)</td>
<td>Le(a+b−) 52</td>
</tr>
<tr>
<td>Heterozygous mutated (Le/le)</td>
<td>208 (41.6)</td>
<td>131 (26.2)</td>
<td>Le(a−b+) 38</td>
</tr>
<tr>
<td>Homozygous mutated (le/le)</td>
<td>39 (7.8)</td>
<td>19 (3.8)</td>
<td>Le(a−b−) 1</td>
</tr>
</tbody>
</table>

* W, wild-type; H, heterozygous mutated; O, homozygous mutated.
methods of determining the Lewis blood group status. If genotyping is considered as the “gold standard”, the sensitivity and specificity of blood grouping for identifying Lewis-negative individuals were 92.3% and 97.8%, respectively.

Three phenotypically Lewis-positive individuals, one Le(a−b−) and two Le(a−b+) were genotyped (double determinations) as le/le, i.e., Lewis-negative. In 10 erythrocyte Lewis-negative individuals, genotyping and blood grouping showed conflicting results. The individuals were blood grouped as Lewis-negative but genotyped as Lewis-positive (nongenuine Lewis-negative individuals). In this group, a significant overrepresentation of heterozygous individuals was observed (P < 0.05), confirming a previously proposed hypothesis on the origin of the nongenuine Lewis-negative phenotype (9). In eight of the individuals, records were available on the ABO histoblood types. Four were A1, two were O, and two were A1B. The data were too few to determine if an overrepresentation of A1 was present, as was found previously among nongenuine Lewis-negative individuals (9). As determined by PCR-cleavage assays, none of the individuals had the mutation C445A, which very seldom is associated with the Lewis-negative phenotype in the Caucasian population (data not shown). The possibility exists, of course, that as yet unidentified mutations are causing the negative phenotype. However, on the basis of previous investigations by us (9) and others (27) this can only be a very rare event.

The results of secretor genotyping of 498 apparently healthy individuals are presented in Table 3. In two individuals, secretor typing could not be performed because DNA was not available. Allele frequencies were 58.4% for the wild-type allele and 41.6% for the mutated allele G428A. There was significant deviation from Hardy-Weinberg proportions for the distributions of the secretor genotypes (P = 0.03). Genotyping of 700 individuals in our laboratory, however, showed that the secretor genotypes were in fact in Hardy-Weinberg equilibrium (data not shown).

The PCR-cleavage assay for the mutation G428A was performed in 90 Lewis-positive individuals who had been blood grouped previously as nonsecretors, Le(a−b−), by agglutination of erythrocytes. G428A was the mutation most responsible for the nonsecretor phenotype in the Danish population. Except for one individual, all were homozygous mutated G428A. This individual was stud-}

**Table 3. Distributions of secretor genotypes and phenotypes.**

<table>
<thead>
<tr>
<th>Genotype (G428A)</th>
<th>Cases (%)</th>
<th>Secretors</th>
<th>Nonsecretors</th>
<th>Le(a−b−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous wild-type (Se/Se)</td>
<td>183 (36.7%)</td>
<td>160</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Heterozygous mutated (Se/se)</td>
<td>216 (43.4%)</td>
<td>200</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Homozygous mutated (se/se)</td>
<td>99 (19.9%)</td>
<td>2</td>
<td>89</td>
<td>8</td>
</tr>
</tbody>
</table>

* Secretor phenotypes were deduced from the Lewis phenotypes of erythrocytes, with Le(a−b−) being secretors and Le(a−b−) being nonsecretors.

ied further by DNA sequence analysis of PCR products of the open reading frame of the secretor allele. The lack of the G428A (Trp143→ter) mutation was confirmed, and two other mutations, C357T and A385T, in homozygous form were identified. A385T in combination with C357T previously has been associated with the nonsecretor phenotype in Japanese people (10) but also with the Le(a−b+) partial-secretor phenotype in Indonesians, Taiwanese, and Polynesians (11, 15, 16). Two individuals were blood grouped as secretors; however, genotyping showed that they were homozygous mutated for G428A.

The kappa measurement of agreement was calculated as 0.96, which indicates that there is good agreement between the two methods of determining the Lewis blood group status in secretor-positive individuals. However, conventional blood grouping could not determine the secretor status of the 46 individuals who were blood grouped as Lewis-negative.

On the basis of the above results, we consider PCR-based genotyping a more reliable method for determining secretor and Lewis histo-blood groups.

**Determination of CA 19-9**

To study the short-term CVI for CA 19-9 in serum, the same protocols were performed on three occasions 1 week apart in 13 Lewis- and secretor-positive individuals; specimens were assayed once. The short-term CVI was 15.8% (95% confidence interval, 10.1–31.3%). The CVA, determined from the results of 10 replicates of three samples in a single assay, was 9.8%. The data on variability are shown in Table 4 along with values from a previously published study of CA 19-9 variability (28).

CA 19-9 was measured in 497 individuals. In three individuals, no serum was available. The test for outliers identified two points, which were removed. CA 19-9 could not be detected in any of the Lewis-negative indi-

**Table 4. Variability and imprecision analysis for CA 19-9.**

<table>
<thead>
<tr>
<th></th>
<th>Present study</th>
<th>Plebani et al. (28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean concentration, kilounits/L</td>
<td>6.8</td>
<td>17.1</td>
</tr>
<tr>
<td>CVα, %</td>
<td>10</td>
<td>2.7</td>
</tr>
<tr>
<td>CVb, %</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>CVg, %</td>
<td>102</td>
<td>159</td>
</tr>
<tr>
<td>Index of individuality</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Critical difference, %</td>
<td>51.5</td>
<td>44.6</td>
</tr>
<tr>
<td>Index of heterogeneity</td>
<td>0.83</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Individuals (39 individuals). The data for CA 19-9 for all individuals and the different subgroups did not follow a gaussian distribution; therefore, nonparametric statistical methods were used for distribution analyses.

Women were found to have significantly higher concentrations of CA 19-9 than men \((P < 0.01)\). The mean age for women was 46.1 years (range, 21–69 years); for men, the mean age was 36.8 years (range, 21–66 years). No statistical differences were observed, however, when the data for men and women were divided separately into four age groups (<30 years, 30–40 years, 40–50 years, and >50 years). The cause of this sex difference remains unexplained.

On the basis of secretor and Lewis genotyping, all Lewis-positive individuals were divided into six different groups. The distribution of CA 19-9 in these six groups is shown in Fig. 2. Genotype groups were not subgrouped according to sex because the differences in medians were small and the sample sizes would be too small to derive valid data.

In accordance with previous publications, secretors (genotype groups 3–6) had less CA 19-9 in their serum than nonsecretors (genotype groups 1 and 2; \(P < 0.05\)). Also in agreement with previous findings, significant differences between the Lewis homozygous wild-type individuals (genotype groups 1, 3, and 5) and heterozygous individuals (genotype groups 2, 4, and 6) could be observed for both secretors and nonsecretors (genotype groups 1 and 2, \(P = 0.04\); genotype groups 2 and 3, \(P < 0.01\); and genotype groups 5 and 6, \(P < 0.01\)).

Our group and others have hypothesized previously that, in addition to the Lewis genotype, CA 19-9 concentrations in secretor individuals would also depend on the secretor genotypes \((Se/Se \text{ or } Se/se)\). As expected, individuals who were heterozygous mutated in the secretor gene (genotype groups 3 and 4) had higher concentrations of CA 19-9 in serum than individuals who were homozygous wild type (genotype groups 5 and 6; borderline significant for individuals who were homozygous wild type in the Lewis gene: \(P = 0.08\) and 0.01).

Reference values for CA 19-9 for subgroups with different secretor and Lewis genotypes were determined by nonparametric methods as described by Solberg (29) (Table 5). The upper reference limit for all individuals was 28.7 kilounits/L; for secretors and nonsecretors the upper limits were 12.4 and 61.2 kilounits/L, respectively. Partitioning of the data as described by Harris and Boyd (30) was justified by the finding that a statistical difference between the different subgroups was found and that partitioning reduced between-person variability. Stratification according to secretor status seemed especially valuable because the difference between subgroup means (76%) exceeded 25% of the reference range for the combined population.

We consider the use of genotype-based reference intervals for CA 19-9 as one possible way to increase the clinical utility of this tumor marker.

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**Table 5. Genotype-specific reference values for CA 19-9 in serum.**

<table>
<thead>
<tr>
<th>Genotype groups</th>
<th>Lewis gene</th>
<th>Secretor gene</th>
<th>n</th>
<th>Median, kilounits/L</th>
<th>Range, kilounits/L</th>
<th>Upper reference limit, b kilounits/L</th>
<th>CV%, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All individuals</td>
<td></td>
<td></td>
<td>497(^a)</td>
<td>4.1</td>
<td>&lt;2.5–66.5</td>
<td>28.7</td>
<td>102.2</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td>118</td>
<td>5.2</td>
<td>&lt;2.5–47.2</td>
<td>30.2</td>
<td>94.2</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td>379(^a)</td>
<td>3.6</td>
<td>&lt;2.5–66.5</td>
<td>23.0</td>
<td>106.9</td>
</tr>
<tr>
<td>1 Le/Le</td>
<td>Se/se</td>
<td></td>
<td>51</td>
<td>14.4</td>
<td>2.9–61.2</td>
<td>50.6</td>
<td>69.4</td>
</tr>
<tr>
<td>2 Le/le</td>
<td>Se/se</td>
<td></td>
<td>39</td>
<td>12.2</td>
<td>2.6–30.0</td>
<td>30.0</td>
<td>45.9</td>
</tr>
<tr>
<td>3 Le/Le</td>
<td>Se/se</td>
<td></td>
<td>113</td>
<td>4.7</td>
<td>&lt;2.5–27.2</td>
<td>17.7</td>
<td>68.8</td>
</tr>
<tr>
<td>4 Le/le</td>
<td>Se/se</td>
<td></td>
<td>91</td>
<td>3.7</td>
<td>&lt;2.5–12.1</td>
<td>11.0</td>
<td>48.2</td>
</tr>
<tr>
<td>5 Le/Le</td>
<td>Se/Se</td>
<td></td>
<td>86</td>
<td>3.7</td>
<td>&lt;2.5–28.7</td>
<td>27.6</td>
<td>93.1</td>
</tr>
<tr>
<td>6 Le/le</td>
<td>Se/Se</td>
<td></td>
<td>77</td>
<td>3.0</td>
<td>&lt;2.5–10.2</td>
<td>7.9</td>
<td>42.9</td>
</tr>
</tbody>
</table>

\(^a\) Upper reference limit is nonparametrically calculated 0.975 fractile.

\(^b\) In three individuals, no serum was available.
Discussion

The data for CA 19-9 for all individuals and the different subgroups were not gaussian distributed, and therefore nonparametric statistical methods were used for distribution analyses. It is remarkable that the upper reference limits for the group with the highest concentration in healthy individuals (Le/Le and se/se) and the group with the lowest concentration (Le/le and Se/Se) deviated sixfold. For example, a CA 19-9 value of 50 kilounits/L would be sixfold the upper reference limit value in group 6, whereas it would be regarded as within the reference interval for an individual belonging to group 7, with the highest concentrations (Le/Le and se/se).

The upper 0.975 fractile limit of the reference range for CA 19-9 for all individuals was established as 28.7 kilounits/L (Table 5). The cause of the deviation from the previously established upper reference limit of 37 kilounits/L is not known; however, the use of different methodologies seems to be the most probable explanation. The distribution of secretors and nonsecretors and the frequency of Lewis-negative individuals (7.8%) in these data did not differ from the distribution in the population at large. This finding, however, stresses the importance of establishing a reference range for the individual methodology.

Except for analytical variation, the calculated values for CV_A, CV_V, and CV_C are comparable to those reported by Plebani et al. (28). The index of individuality was low (0.15), which suggests that CA 19-9 has limited usefulness in detecting disease-associated changes when used in conjunction with conventional reference intervals. For follow-up purposes, individuals are their own references, and the clinician is mainly interested in increases or decreases of the concentration of the tumor marker. Population-based reference values are interesting, however, when after initial treatment, a high marker concentration approaches the upper reference limit. On the basis of the genotype-interpreted reference limits, it seems possible to conclude whether the marker has reached a concentration where one can expect an absence of tumor tissue.

We have shown previously that a gene-dosage effect exists for mutations in the Lewis gene, because heterozygous individuals have less CA 19-9 in their serum and urine, as do homozygous wild-type individuals (9, 22). With this study we have also shown a gene-dosage effect of the Caucasian secretor mutation, G428A, in Lewis heterozygous individuals (Le/le): the serum concentrations of CA 19-9 in FUT2 heterozygous (Se/se) individuals were higher than the concentrations in homozygous wild-type individuals (Se/Se; P = 0.01). This subject has been dealt with in another recent study concerning Asian mutations in the Lewis and secretor genes (31). Our results are difficult to compare with the data in the latter study because of the differences in the ethnic groups and because we used nonparametric methods to determine distributions.

Knowledge of within-person variation and the critical difference are important when CA 19-9 is used for follow up. The calculated critical difference (at P = 0.05) between two serial measurements of 51.5% indicates that relatively large changes in CA 19-9 are required before one can feel certain that the change is significant. It should be emphasized that the observed variation is based on CA 19-9 measurements in the serum of healthy individuals. The variation in CA 19-9 values in cancer patients with very high marker concentrations cannot be predicted from the present data, however, because very high serum concentrations may have different elimination mechanisms than health-related concentrations.

The determination of Lewis and secretor histo-blood group status has until now relied on immunological reactivities with Lewis carbohydrate antigens on erythrocytes, complemented with determination of the presence or absence of ABH antigen in the saliva of Lewis-negative individuals by the hemagglutination inhibition test.

Previously, we identified nongenuine Lewis-negative individuals as being either heterozygous mutated in the Lewis gene, which leads to a weak phenotype, or A1 individuals. In the latter case, the problem is associated with the blood grouping of erythrocytes in blood banks. A1 is the result of a highly reactive blood group A transferase, which converts most of the H trisaccharide to A tetrasaccharide on the erythrocyte surface (32, 33). Because of this, only a few cells will have unmasked Lewis structures on the cell membrane, and many will be A1 Lewis b or simply A1. The structure A1 Lewis b is more difficult to detect with anti-Lewis b antibodies than the structure Lewis b because of masking by the N-acetyl-

p-galactosamine added on by the blood group A transferase. In three individuals, a discrepancy was found between Lewis genotypes that were negative (le/le) and an agglutination test in the blood bank that was Lewis-positive. An explanation for this could be that the antibodies used for agglutination have been cross-reacting with irrelevant epitopes on the surface of erythrocytes. Our inclination is thus to regard genotyping as the gold standard method because it is able to identify minor errors in agglutination-based phenotyping. Secretor and Lewis genotyping represents a reliable method for determination of secretor and Lewis type on the assumption that a homogeneous population is tested. Furthermore, for Lewis-positive individuals genotyping yields information on the specific allelotype, which is an aspect that has been shown to be important for the expression of the final carbohydrate epitopes of these two glycosyltransferase genes.

Previous epidemiological studies have shown nonsecretor status and a Lewis-negative blood group to be genetic markers of different disease states (34, 35). In future studies, determination of blood groups on the basis of genotyping can replace the need for fresh blood and saliva samples because Lewis and secretor genotyping...
can be performed on blood spots or on DNA from archival material such as paraffin-embedded tissue.

CA 19-9 in serum is used to support the diagnosis of cancer, especially in the pancreas and the colon (18). Furthermore, this marker can be used for follow up of these patients after initial treatment (19–21). In terms of diagnosis, it is highly relevant to establish a reliable upper reference limit above which disease must be suspected. In the present study, we showed a highly significant difference in the upper reference limits for CA 19-9 in serum, based on grouping of individuals according to their secretor and Lewis genotypes.

In conclusion, we suggest that when CA 19-9 in serum is used for diagnosis of cancer it should be interpreted on the basis of a patient’s secretor and Lewis genotype. This may seem a laborious procedure, but one has to consider that it is a once-only test that, because of continuous advances in PCR technology, probably will be adapted for automation. On the basis of the calculation of a critical difference for sequential values (significant at $P \leq 0.05$) of 51.5%, a 40–50% change in marker concentration is suggested as limit for significant change when the tumor marker is used for follow up. PCR-based genotyping is an accurate and convenient method to determine secretor and Lewis histo-blood groups.

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