In summary, for patients we can obtain a more approximate value for LDL-C that, when the serum concentrations of TG are >3.3 mmol/L, is almost surely overestimated. Only those with an estimated LDL-C >4.1 mmol/L, especially if their serum TG is <3.3 mmol/L, will merit additional investigation. This proposed approach is summarized in Figure 2.

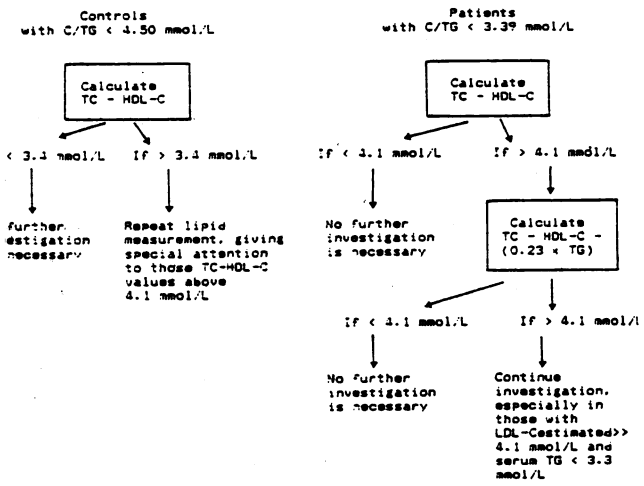

Fig. 2. Strategy proposed to determine if further investigation is necessary among those individuals having a possibly unreliable calculated LDL-C

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Quantitative Nephelometric Assay for Determining Myoglobin Evaluated

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A recently introduced automated nephelometric immunoassay involving shell/core particles for determination of myoglobin (Behringwerke) was evaluated with the BNA Nephelometer. Method precision was good: the intra-assay CV varied between 1.5% and 6.1%; with daily calibration, the interassay CV ranged between 1.5% and 7.5%. For usual sample dilutions, the assay response varied linearly with myoglobin concentrations up to 23.1 nmol/L. After automatic dilution by the instrument, concentrations up to 2310 nmol/L could be measured without high-dose "hook" effect. Further manual dilution allowed measurement of myoglobin concentrations up to 26 000 nmol/L. Calibration was stable for at least seven days. We detected no significant interferences from hemoglobin, haptoglobin, bilirubin, iodine-containing contrast media, and rheumatoid factors. Treating lipemic samples with Lipoclean (Behringwerke) decreased test results. Simultaneously drawn serum and plasma samples from the same subject showed no consistent differences in myoglobin concentrations. The mean reference myoglobin concentration was 1.380 (SD 0.82) nmol/L for men and 0.878 (SD 0.45) nmol/L for women. In patients with renal insufficiency, serum creatinine values were moderately related to serum myoglobin values ($r = 0.465$). Although a commercial radioimmunoassay (Byk-Sangtec) and the nephelometric assay intercorrelated well ($r = 0.929$), values obtained by nephelometry were significantly lower ($P < 0.05$). By both assays, results for heart and skeletal muscle tissue extracts showed no correlation, a finding that suggests the existence of multiple forms of myoglobin in human tissues. We conclude that immunonephelometry is a rapid, practical, and reliable method for measuring myoglobin in serum.

Additional Keyphrases: *reference values · renal insufficiency · sex- and age-related effects*

Myoglobin is a small-molecular-mass oxygen-binding protein (M_r 17 700), abundant in human skeletal and

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cardiac muscle. Determination of myoglobin concentrations in serum is therefore useful for evaluation of skeletal muscle damage (*1*), for early diagnosis and monitoring of acute myocardial infarction (AMI) (*1–3*), and for detecting coronary reperfusion (*4*) or reinfarction (*1*). Measuring cumulative myoglobin release in serum has been proposed for sizing infarcts (*5*).

In the routine clinical laboratory, myoglobin concentrations can be determined by radioimmunoassays (RIAs) (*6*) or by latex agglutination tests (*7*). However, RIAs are time consuming and therefore not suited for emergency testing. Latex agglutination tests give only semiquantitative results and can occasionally give false-negative results in the presence of antigen excess ("hook" effect). Because serum myoglobin concentrations in clinical practice are in the range 0.5–50 000 nmol/L, simultaneous serial serum dilutions must be measured. In the present study, we evaluated a recently introduced automated nephelometric method based on shell/core polymer particles coated with anti-myoglobin antibodies and compared results with those obtained with a commercially available RIA. Here we present the results of this analytical evaluation, which was performed in two university hospitals.

Materials and Methods

Methods

Nephelometric method. In each center, myoglobin in serum, plasma, and tissue was assayed with an automated nephelometric immunoassay (NA-Latex Myoglobin Test; Behringwerke, Marburg, F.R.G.) based on shell/core particles coated with anti-myoglobin antibodies (*8*), with use of a selective multi-protein Behring Nephelometer Analyzer. In one center (A; Universitair Ziekenhuis, Gent), the analyzer had been intensively used for three years. The other center (B; Centre Hospitalier Universitaire, Liège) used a new analyzer. In both cases, the assay was performed according to the manufacturer's procedure, with a standard serum dilution of 1:20, unless otherwise stated, and a 12-min incubation time for the antigen–antibody reaction. We used a commercial myoglobin control serum from Behringwerke.

Comparison study. For comparison, we assayed myoglobin with an RIA (RIA-mat myoglobin no. 323.500 (3830); Byk-Sangtec, Dietzenbach, F.R.G.), performed according to the package insert. We assayed 127 serum samples (40 from healthy blood donors, six from renal insufficiency patients, 55 from acute myocardial infarction patients, 22 various samples with increased myoglobinemia, four control sera) and 29 tissue extracts, made from human heart (n = 15) and skeletal muscle (n = 14) tissue samples obtained at autopsy. Tissue extracts were prepared according to Tsung (*9*).

Stability of the analyte. We evaluated the stability of the analyte in serum by measuring myoglobin concentration in various serum sample pools before and after storage at different temperatures: at room temperature (for two days), refrigerated (4 °C for 10 days), and frozen (−21 °C for 60 days).

Stability of calibration. To assess the stability of calibration of the nephelometric assay, we compared for each instrument the test results obtained with use of stored and daily calibration curves.

Imprecision. Nine different serum pools (P1–P9) were prepared from serum samples with increased myoglobin concentrations (>4.7 nmol/L). We tested intra-assay reproducibility with a series of 20 aliquoted samples from the different pools. We also pooled serum made from sample from patients with AMI, or with liver or renal insufficiency. Interassay reproducibility was evaluated by assaying serum pools and the control serum during nine to 15 consecutive days.

Sensitivity and linearity. We tested the sensitivity of the nephelometric assay for serum in both standard and sixfold dilution, as provided by the instrument. To evaluate the linearity of the nephelometric assay, we used serial automated or manual dilution of serum samples from five patients with rhabdomyolysis, which contained high concentrations of myoglobin in serum (575–28 000 nmol/L).

Interference studies. We studied the effects on myoglobin test results of adding to serum pools various potentially interfering substances: purified human haptoglobin (phenotypes 1-1, 2-1, and 2-2, nos. H-0138, H-9887, and H-9762 respectively; Sigma Chemical Co., St. Louis, MO 63178) and water-soluble iodine-containing roentgenographic contrast media (Omnipaque; iodine content: 240 and 350 g/l; Nycomed, Oslo, Norway). Effects of triglycerides, hemoglobin, bilirubin, and rheumatoid factor were studied by adding to samples serum that was enriched in these compounds up to final concentrations of 13.7 mmol/L, 0.17 mmol/L, 547 μmol/L, and 2500 int. units/L, respectively. Lipid extraction of serum samples (n = 39) by means of the commercial extraction agent Lipoclean (Behringwerke) was carried out according to the manufacturer's recommendations (three volumes of extraction medium for two volumes of serum).

Blood Samples

Serum and EDTA- or citrate-treated plasma samples were centrifuged (1000 × *g*, 10 min, room temperature) and analyzed within 24 h after venipuncture.

Patients

Apparently healthy blood donors (197 men, mean age ± SD: 39.9 ± 13.0 years, and 85 women, 39.8 ± 12.1 years) served as a reference population for determining the normal range of myoglobin in serum. Serum samples from 97 patients with renal insufficiency (47 men, 44 women, age 55.3 ± 16.3 years; serum creatinine and urea concentrations of 875 ± 424 μmol/L and 27.56 ± 7.80 mmol/L, respectively), were used to test the effect of glomerular filtration rate on the myoglobin concentration in serum.

Results

Reproducibility. Intra-assay coefficients of variation (CV) for patients' and control samples were between 1.5% and 6.1%; daily calibrated interassay CVs were between 1.5% and 7.5%. Lowest CV values were obtained with the new equipment. Table 1 summarizes intra- and interassay CV for different pools used in the evaluation centers.

Sample stability. Storage of serum samples at room temperature for 48 h, refrigerated at 4 °C up to one week, or frozen during 60 days did not affect myoglobin value significantly.

Stability of calibration. To compare the stability of daily and single calibration of myoglobin tests, we repeatedly measured serum pools and a control. Using a single calibration curve during the entire period of interassay reproducibility testing, we observed an increase in the imprecision in the two centers: in hospital A (pools 7–9), CV increased to 6.8–14.8% (vs 5.9–7.5% with a new calibration each day); in hospital B (pools 4–6), CVs increased

Table 1. Reproducibility of Nephelometric Myoglobin Determination

	Center A				Center B			
Intra-assay (n = 20 each)								
	P1[a]	P2	P3	C	P4	P5	P6	C
Mean, nmol/L	6.64	6.13	6.05	5.99	4.57	9.10	17.24	5.82
SD, nmol/L	0.41	0.13	0.16	0.19	0.09	0.22	0.50	0.08
CV, %	6.1	2.1	2.7	3.2	2.0	2.4	2.9	1.5
Interassay (with daily calibration)								
	P7	P8	P9	C	P4	P5	P6	C
No. of days	9	9	9	13	13	13	13	13
Mean, nmol/L	8.13	10.40	18.05	5.83	4.74	8.79	17.34	5.67
SD, nmol/L	0.59	0.61	1.36	0.34	0.15	0.19	0.27	0.24
CV, %	7.2	5.9	7.5	5.8	2.2	2.1	1.5	4.2
Interassay (with single, stored calibration)								
	P7	P8	P9	C	P4	P5	P6	C
No. of days	9	9	9	9	15	15	15	15
Mean, nmol/L	7.34	10.07	15.98	5.07	5.07	9.16	17.66	6.11
SD, nmol/L	0.74	0.68	2.41	0.45	0.25	0.38	0.88	0.33
CV, %	10.2	6.8	14.8	8.9	4.8	4.2	5.0	5.4

[a] P1–P9: different serum pools; C, control serum.

4.2–5.0% (vs 1.5–2.2% with a new calibration each day). Table 1 summarizes results of calibration stability on various serum pools and controls.

Range and linearity. The basic measuring range covers myoglobin concentrations from 1.39 to 22.4 nmol/L. When sample dilution was sixfold, concentrations as low as 0.34 nmol/L could be detected. Samples with high myoglobin concentration can automatically be rerun by the analyzer by further sample dilution up to 1:100, 1:400, and 1:2000. In this way, we found the standard curve of the nephelometric method to be linear from 0.34 to about 2310 nmol/L. A high-dose "hook" effect does not occur in the tested range (myoglobin concentrations up to 26 000 nmol/L).

Interferences. No interference from hemoglobin (up to a final concentration of 0.178 mmol/L) and haptoglobin (up to a final concentration of 6.7 g/L) could be detected. Also, addition of solutions of the three different phenotypes (1-1, 2-1, and 2-2) of purified human haptoglobin (final concentration 0.5 g/L) to serum samples did not influence test results. Addition of bilirubin (final concentrations up to 547 μmol/L) and rheumatoid factor (final concentrations up to 2500 int. units/L) to serum pools with increased myoglobin concentrations did not interfere with the nephelometric assay. The presence of iodine-containing contrast media (Omnipaque) up to a final iodine concentration of 24 g/L in the patients' sera did not interfere with the assay, except in one out of 10 cases. However, the concentrations used exceed the maximal iodine concentrations usually obtained during coronarography (±15 g/L). Effects of hypertriglyceridemia on test results were negligible up to concentrations of 13.7 mmol/L.

Lipid extraction. Lipid extraction of normolipemic serum samples resulted in a variable relative loss [23.3 (±20.5)%, median 38%, n = 36] of myoglobin concentration. In hyperlipemic samples, loss of myoglobin concentration after lipid extraction was more pronounced [81.2 (±3.7)%, n = 3]. Addition to normolipemic serum of myoglobin-poor serum rich in triglycerides (final triglyceride concentration of 6.84 mmol/L) resulted in an increased myoglobin loss. Therefore, use of lipid extraction procedures before nephelometric determination of myoglobin is not recommended.

Comparison between serum and plasma test results. Myoglobin values in simultaneously drawn serum and EDTA-plasma samples showed a good correlation: y (EDTA-plasma myoglobin, nmol/L) = 0.665x (serum myoglobin, nmol/L) + 1.757 (r = 0.956, n = 19, S_{yx} = 2.89). However, for extremely low and high myoglobin concentrations, relative differences between serum and EDTA-plasma concentration may be high. Similar findings were obtained when serum was compared with citrate-treated plasma: y (citrate-treated plasma myoglobin, nmol/L) = 1.18x (serum myoglobin, nmol/L) + 2.22 (r = 0.775, n = 10, S_{yx} = 6.80).

Correlation with RIA-method. Myoglobin was simultaneously determined by both methods in a group of 127 serum samples from the reference population (n = 40), renal insufficiency (n = 6), and AMI patients (n = 55). A good correlation between the nephelometric and RIA assays for serum myoglobin was obtained: log[y (myoglobin-RIA, nmol/L)] = 0.944log[x (myoglobin-nephelometry, nmol/L)] + 0.012 (r = 0.929, n = 127, S_{yx} = 0.205). However, in both skeletal and heart muscle tissue samples, correlation between both methods was lacking.

Reference values. In the reference population, values for males (1.35 ± 0.81 nmol/L) were significantly (P <0.05) higher than those for females (0.86 ± 0.44 nmol/L). In both

Table 2. Reference Values (Mean ± SD) for Serum Myoglobin According to Age and Sex

Age group, y	Males		Females	
	n	nmol/L	n	nmol/L
11–20	4	1.297 ± 0.105	4	0.503 ± 0.134[a]
21–30	39	1.162 ± 0.531	18	0.751 ± 0.299[b]
31–40	80	1.325 ± 0.804	28	0.796 ± 0.394[b]
41–50	42	1.440 ± 0.852	15	1.054 ± 0.559[a]
51–60	17	1.436 ± 0.649	16	1.075 ± 0.569[a]
61–70	15	2.046 ± 1.497	4	1.004 ± 0.553[a]

[a] P <0.05, [b] P <0.01. Differences between sex groups were evaluated by a Mann–Whitney U test.

sexes, reference values increased significantly with age. Table 2 depicts reference values for serum myoglobin concentration according to sex and age. Patients with renal insufficiency also showed significantly higher values. Values for serum myoglobin concentration in patients with various degrees of renal insufficiency are given in Table 3. In these patients, concentrations of serum creatinine and serum myoglobin correlated moderately: y (myoglobin, nmol/L) = $0.00587x$ (creatinine, μmol/L) + 3.22 (r = 0.465, n = 91, $S_{y\cdot x}$ = 6.314).

Discussion

The new nephelometric latex myoglobin test allows fast and convenient myoglobin determinations of high analytical quality, with use of only small amounts of serum (80 μL). These properties make the method suited for delivering stat results. Intra- and interassay CVs are within acceptable limits. Assay results are available within 12 min, which makes the method suited for the emergency laboratory. Samples with high myoglobin concentration (>23.1 nmol/L) can be rerun automatically by the instrument from a higher predilution, which is an advantage with respect to RIA methods. Manual dilution of the serum sample was necessary in only one case, where serum myoglobin concentrations exceeded 139.5 nmol/L. In the observed concentration range (0–26 000 nmol/L), no high-dose "hook" effect could be detected, which is advantageous over latex agglutination tests, where false-negative results may be obtained owing to excess antigen (*10*). Daily calibration of the nephelometric assay resulted in lower interassay CV values. Calibration curves remained stable during at least one week. Reference values for serum myoglobin concentration obtained by nephelometry are lower than those obtained by RIA (*11, 12*). In agreement with literature, the reference values we obtained for serum myoglobin are age- and sex-related and positively correlated with serum creatinine concentration (*11, 12*). Interferences resulting from the presence of rheumatoid factors, hemoglobin, bilirubin, and triglycerides are negligible under routine conditions. Moreover, iodine-containing contrast media, often used in invasive procedures for evaluating and treating AMI, do not interfere with the nephelometric assay. Lipid extraction, which leads to an underestimation of the myoglobin concentration, is not recommended for turbid samples. With respect to Byk-Sangtec RIA results, good correlation was obtained for serum and plasma samples. However, values obtained by the automated nephelometric assay are about 30% lower than those obtained by RIA.

In the tissue samples, a striking lack of correlation between both assays was observed. These results may indicate differences between tissue and serum forms of myoglobin and demonstrate heterogeneity of antigenic sites of human myoglobin, which is in agreement with earlier findings on human serum myoglobin (*13*) and animal myoglobin (*14, 15*). Although the correlation between serum and plasma myoglobin concentrations was good important differences may be found at higher concentrations (increased values for citrate-treated plasma, decreased values for EDTA-plasma).

In conclusion, immunonephelometric determination of serum myoglobin is a fast, convenient, and reliable method appropriate to the emergency laboratory.

Table 3. Effect of Glomerular Filtration Rate on Myoglobin Concentration in Serum in Renal Insufficiency

Serum creatinine concn, μmol/L (range)	n	Serum myoglobin concn, nmol/L[a] (mean ± SD)
180–450	20	3.75 ± 1.45
450–700	11	6.53 ± 3.92
700–950	17	7.80 ± 4.62
950–1200	19	9.99 ± 6.65
1200–1500	12	11.90 ± 6.41
>1500	12	11.73 ± 6.53

[a] All significantly different (P <0.01) from the reference population values (Mann–Whitney U test).

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