food high in purines, serum concentrations were 58.2 (18.5) \(\mu\)mol/L for hypoxanthine, 11.1 (3.4) \(\mu\)mol/L for xanthine, and 486 (81) \(\mu\)mol/L for uric acid, indicating that serum hypoxanthine and xanthine concentrations were also affected by intake of purine-containing foods.

The concentrations in allopurinol-treated patients were similar to those in untreated patients, but both treated and untreated gout patients had lower uric acid and higher hypoxanthine and xanthine concentrations than did allopurinol-treated hyperuricemic patients. The differences in hypoxanthine and xanthine concentrations in gout patients compared with patients with asymptomatic hyperuricemia were not as large in treated as in untreated patients. Allopurinol-treated patients had lower uric acid, xanthine, and hypoxanthine concentrations, as expected from the action of allopurinol on purine metabolism. Other nucleosides and deoxynucleosides also differed to some degree in the 2 patient groups, although not as markedly as hypoxanthine and xanthine. Hypoxanthine and xanthine can thus be regarded as marker compounds for gout diagnosis.

The HPLC-UV-MS/MS method we describe is specific, simple, and inexpensive, requiring only a small volume of serum (100 \(\mu\)L) and easy sample preparation. Use of auto-MS/MS avoided errors in peak identification.

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References

Ischemia-Modified Albumin Measurements in Symptom-Limited Exercise Myocardial Perfusion Scintigraphy Reflect Serum Albumin Concentrations but Not Myocardial Ischemia

The albumin cobalt-binding test (Ischemia Technologies), which measures the concentration of ischemia-modified albumin (IMA), has been cleared by the US Food and Drug Administration for use as a rule-out marker for acute myocardial ischemia (1). The test is based on the reduced capacity of human albumin to bind cobalt as a result of structural changes in the NH2 terminus of the albumin molecule in conditions of myocardial ischemia (2, 3). Moreover, IMA concentrations correlated with disease severity in systemic sclerosis (4), and an exercise-induced decrease in IMA concentrations correlated with the ankle-brachial index in patients with peripheral artery sclerosis (5). Little is known, however, about the relationship between exercise-induced myocardial ischemia, albumin kinetics, and IMA kinetics. We therefore investigated this relationship in 38 patients with chest complaints and suspected coronary artery disease who were undergoing symptom-limited exercise myocardial perfusion scintigraphy. Myocardial perfusion scintigraphy was performed according to the guidelines of the American Society of Nuclear Cardiology (6), with a 2-day stress/rest protocol. A dose of 500 MBq of \([99mTc]\)-Tetrofosmin was administered at rest and at peak exercise. Electrocardiogram-gated single photon emission tomography imaging was started 45 min to 1 h after the administration of radioactive-labeled tracer. All patients exercised on a bicycle ergometer with a starting workload of 50 W, increasing every 2 min by 25 W. Endpoints for exercise included achievement of at least 85% of the age-predicted heart rate, recognizable chest pain, and a >2-mm ST-segment depression (7). All patients fasted both days. All antiangiinal medication was discontinued for at least 48 h before the exercise test and restarted immediately after exercise.

Stress and rest perfusion images were scored in consensus by two experienced nuclear medicine physicians (H.J.V. and B.L.F.V.E-S.), who used a 5-point semiquantitative score for each of 17 myocardial segments. Perfusion defect severity was classified as normal (0), equivocal abnormal (1), mildly abnormal (2), moderately abnormal (3), or severely abnormal (4). The summed stress score (SSS), summed rest score (SRS), and the difference between those scores [summed difference score (SDS)] were then calculated. Improvement at rest of one or more grades was considered to be a “reversible” perfusion
defect if present in more than one adjacent segment. An SDS ≥3 was considered to indicate clinically relevant ischemia. Estimates of left ventricle ejection fraction (LVEF) were calculated by use of a completely automated algorithm that has been described previously and validated (8, 9).

On the day of the symptom-limited exercise test, serum samples were drawn before exercise, at maximum exercise, and 1, 2, 3, 4, 5, and 6 h after exercise; all samples were then frozen at −80 °C until analysis. A Roche Diagnostics Modular P-800 instrument was used for spectrophotometric measurement of IMA (ACB test; Ischemia Technologies), turbidimetric measurement of serum IgM (Tina Quant, IgM gen 2; Roche Diagnostics), and photometric measurement of serum creatinine (Crea plus; Roche Diagnostics) and albumin (Tina Quant; Roche Diagnostics). Creatinine clearance was calculated by use of the Cockcroft–Gault formula.

The χ² test was used to compare dichotomous variables. Continuous variables were compared between groups with the Student t-test or Mann–Whitney U-test as appropriate. The Spearman rank correlation test was used to assess the relationship between nongaussian-distributed continuous variables, and stepwise linear regression analysis was used for multivariate analysis of continuous variables. P values <0.05 were considered to indicate statistically significant differences except for multivariate analysis, for which a P value <0.1 was considered to indicate statistically significant differences. The study was approved by the medical ethics committee.

Of 38 patients studied, 15 had ischemia on myocardial perfusion scintigraphy. Ischemia patients [SDS = 6 (interquartile range, 5–10)] had a higher incidence of documented coronary artery disease and had higher nitrate and statin use than did patients without myocardial ischemia. When we compared results for patients with ischemia vs those without ischemia, we found no differences in male sex [12 (80%) vs 17 (74%); P = 0.67], age [median (interquartile range), 59 (52–72) vs 61 (55–71) years; P = 0.96], SRS [4 (0–11) vs 2 (0–7); P = 0.42], LVEF [51 (47–59)% vs 59 (52–65%); P = 0.14], creatinine clearance [96 (90–125) vs 87 (75–106) mL/min; P = 0.24], and baseline concentrations of albumin [42 (41–46) vs 43 (41–45) g/L; P = 0.66], IgM [0.83 (0.61–1.23) vs 1.00 (0.64–1.40) g/L; P = 0.41], and IMA [99 (92–106) vs 97 (92–104) kilounits/L; P = 0.48]. Baseline IMA concentrations were associated with LVEF (r = −0.538; P = 0.001) and albumin [IMA (kilounits/L) = −2.36 × albumin (g/L) + 200 kilounits/L; r = −0.831; P <0.001; Fig. 1A], but not with any other clinical or biochemical characteristics. Multivariate linear regression analysis showed that the albumin concentration was the only independent predictor of IMA concentration (P <0.001; adjusted R² = 0.776). The relative changes in IMA for patients with or without myocardial ischemia are shown in Fig. 1B. Absolute and relative concentrations of both IMA and albumin did not differ between the groups at any of the time points. At maximum exercise, patients from both groups had IMA concentrations significantly lower than baseline values [median (interquartile range), 98 (92–105) kilounits/L for nonischemic patients and 81 (74–88) kilounits/L for ischemic patients; P <0.001] and significantly higher albumin [43 (41–45) and 46 (44–49) g/L; P <0.001] and IgM [as an indicator of water shift across the vessel wall during exercise; 0.84 (0.62–1.35) and 0.94 (0.69–1.51) g/L; P <0.001] concentrations. The absolute change in IMA correlated with the absolute change in albumin (r = 0.484; P = 0.002) and IgM (r = 0.369; P = 0.027) concentrations. Multivariate linear regression analysis showed that the absolute increase in albumin concentration was the only independent predictor of absolute decrease in IMA (P = 0.003; adjusted R² = 0.204).

Physical exercise causes hemoconcentration, with subsequent increases in concentrations of plasma proteins, such as albumin (10). In a population of persons with suspected myocardial ischemia, we found that IMA, measured by the ACB test, which quantifies the nonbound portion of a fixed amount of cobalt added to albumin in the sample, is specifically dependent on the concentration of serum albumin and does not reflect the presence of myocardial ischemia. Although proponents of the test argued that IMA is a useful diagnostic tool insensitive to albumin concentrations (1), the correlation between IMA and albumin in our study was highly comparable to that found by others (11). Furthermore, the decrease in IMA concentrations after short exercise has also been reported after long-time exercise (i.e., after marathon running) (12). Previous results on the diagnostic value of high IMA...
concentrations in patients with chest pain (13–17) are, at least partly, attributable to the dependency of IMA on albumin, as low albumin concentrations are associated with a higher risk for adverse cardiac events.

References


2. Nandedkar AK, Hong MS, Friedman F. Co 


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Immunoprecipitation for Rapid Detection of Macroprolactin in the Form of Prolactin–Immunoglobulin Complexes, Johan Schiettecatte,1,* Anne Van Opdenbosch,2 Ellen Anckaert,1 Jean De Schepper,2 Kris Poppe,2 Brigitte Veltkamp,2 and Johan Smits2 (1 Laboratory of Radioimmunology and 2 Department of Endocrinology, University Hospital, Vrije Universiteit, Brussels, Belgium; * address correspondence to this author at: Laarbeeklaan 101, B-1090 Brussels, Belgium; fax 32-2-477-50-60, e-mail Johan.Schiettecatte@az.vub.ac.be)

The 3 major forms of serum prolactin (PRL), identifiable by gel-filtration chromatography (GFC), are monomeric PRL (23 kDa), big PRL (45–60 kDa), and big, big PRL or macroprolactin (150–170 kDa) (1). The common macroprolactin is PRL complexed with human IgG, but aggregates of PRL, some extensively glycosylated, may also occur (2–6).

Macroprolactin has slower serum clearance than monomeric PRL and little or no apparent in vivo bioactivity, probably because it does not cross capillary walls (7, 8). Macroprolactin causes diagnostic confusion in evaluating hyperprolactinemia (9, 10).

Polyethylene glycol (PEG) precipitation is a rapid screening method for macroprolactinemia (11–17), but PEG interference with PRL assays limits its general use. The method, moreover, shows nonspecific precipitation (up to 15%), necessitating use of a gray zone.

We recently validated a screening method based on recognition of the IgG component of macroprolactin by anti-IgG-agarose (18); this method, however, had insufficient PRL assay sensitivity for use in moderate hyperprolactinemia (PRL <1000 mIU/L). A more rapid screening method based on PRL-IgG precipitation with protein A-Sepharose was described recently (19).

We report a simple, rapid method, also based on precipitation of PRL-IgG complexes, with protein G-agarose (PGA) suspension and high IgG binding capacity (20 mg/mL of resin). The protein G polypeptide binds the Fc region of all subclasses of the human IgG molecule and also binds the IgG3 fraction (20). Results with this method were compared with those from GFC and PRL-PEG precipitation.

For GFC, we used the fast FPLC system (Pharmacia) with a Superdex 200 HR10/300 prepacked column (Amersham Pharmacia) (11). Serum (100 μL) was applied and eluted with phosphate-buffered saline (PBS; 0.05 mol/L, pH 7.0) at a flow rate of 0.5 mL/min. The first 5 mL was discarded, and thirty-five 0.5-mL fractions were collected and analyzed for PRL by the automated assay on the Elecsys 2010 analyzer. Macroprolactin was identified as a peak of PRL immunoreactivity (above the Elecsys assay detection limit of 10 mIU/L), eluting between IgA and IgG (fractions 12–20) and before monomeric PRL (fractions 23–30). No distinct peak corresponded to the expected migration of big PRL, but small amounts may have occurred as shoulders of the monomeric PRL peak. Monomeric PRL and macroprolactin were quantified from relative areas under the PRL curve and total PRL results.