7 days, 1.92 for birth within 14 days, and 2.4 for birth within 21 days. The likelihood ratio for a negative result \([1 - \text{sensitivity}/\text{specificity}] \) was 0.59 for birth within 7 days, 0.68 for birth within 14 days, and 0.53 for birth within 21 days. The data in Table 1 are similar to those obtained in previous studies using the ELISA-based assay. Joffe et al. (1) reported a NPV of 99.6% and a PPV of 9.1% for 243 patients who delivered within 7 days with gestational ages between 24 and 35 weeks of gestation. Iams et al. (2) reported a NPV of 99.3% and a PPV of 38.9% for 192 patients who delivered within 7 days with gestational ages between 24 and 34 weeks of gestation. This group also calculated a NPV of 95% and a PPV of 40% for patients delivering within 14 days. Peaceman et al. (3) examined 763 patients with gestational ages of 24–34 weeks and reported a NPV of 99.5% and a PPV of 12.7% for patients who delivered within 7 days and a NPV of 99.2% and a PPV of 16.7% for patients who delivered in within 14 days.

In our study, the NPV of fFN using the TLi system compared well with data from previous reports using ELISA-based assays. For patients who delivered within 7, 14, and 21 days, the NPVs were 96.8%, 93.7%, and 93.7%, respectively.

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References

Macroprolactin Detection by Precipitation with Protein A-Sepharose: A Rapid Screening Method Compared with Polyethylene Glycol Precipitation, Remy Sapin1* and Gilles Kertesz2
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Prolactin (PRL) circulates in serum in three major molecular sizes identifiable by gel-filtration chromatography: monomeric PRL (23 kDa), big PRL (45–60 kDa), and big big PRL or macroprolactin (150–170 kDa). Macroprolactin is mainly a complex of PRL with human immunoglobulins G (IgG) (1–4), but aggregates of PRL may also be present (4). Identification of macroprolactin, which has reduced bioactivity but can be the cause of high PRL values in patient samples, can help resolve diagnostic confusion and avoid expensive investigations and inappropriate treatment. It is generally admitted that all samples showing apparent hyperprolactinemia should be examined for macroprolactin.

The immunoassays used to determine prolactinemia react variously with macroprolactin (5). With high-reacting assays such as the Elecsys® PRL assay from Roche Diagnostics, polyethylene glycol (PEG) precipitation has been validated as a rapid technique to detect macroprolactin (6). However, the PRL PEG precipitation test suffers from high nonspecific precipitation [~14–16% (7, 8)], and results of this test may not be definitive in any case, making it necessary to define a gray zone (6, 9). Comparison of the results of a high-reacting method with those of low-reacting methods, such as the ADVIA:Centaur or ACS:180 PRL assays (Bayer Diagnostics), has also been proposed as a screening method (10). However, results obtained for macroprolactinemic sera have been shown to be sample dependent, particularly with the Bayer assays (7, 11), and the presence of macroprolactin can not be excluded by comparing the results obtained with a high- and a low-reacting method.

Recently, a screening method based on the recognition of the IgG component of macroprolactin by goat anti-human IgG-agarose (binding capacity, 1.5 mg IgG/mL of resin) has been validated with the Elecsys assay (12). This method incorporates a 2-h incubation time and a 20-fold dilution of the serum, which may prevent its use in samples with moderate hyperprolactinemia (<1000 mIU/L). In the present study we describe the application of a simple and rapid method based on precipitation of IgG-PRL complexes by a protein A-Sepharose suspension with high IgG binding capacity (16 mg/mL of resin). Protein A is a polypeptide that binds the Fc region of human immunoglobulin molecules, especially IgG1, IgG2, and IgG4 but only a fraction of IgG3 (13). This method involves a short incubation time and a threefold dilution factor only. Results obtained with this new method were compared with those obtained with the PEG precipitation test.

Prolactin was measured in 116 sera from hyperprolactinemic individuals (PRL >600 mIU/L): 23 men (age range, 12–79 years) and 93 women (age range, 8–90 years). These sera were selected on the basis of their PEG-precipitated PRL values (7–96%) to study the whole range of values. The PRL concentrations in these samples were between 636 and 24 400 mIU/L. The procedures were in accordance with the Helsinki Declaration of 1975 and the subsequent 1996 amendments.

We used the automated Elecsys PRL immunoassay on the Elecsys 2010 analyzer. The PRL standard was the third International Reference Preparation WHO 84/500. PEG precipitation was performed as described previously (8). This method involves a twofold dilution. Results were expressed as the percentage of PEG-precipitated PRL (10)
A serum was considered to be negative, i.e., to contain a low proportion of macroprolactin, when the percentage was <50% and positive when the percentage was >60%. The range between 50% and 60% was defined as border-line and constituted a gray area (10, 14). Ready-to-use protein A-Sepharose suspension, obtained from Immuno-tech Beckman Coulter (Marseille, France), was used to precipitate the hIgG fraction in serum samples, according to the manufacturer’s protocol (15). To this end, 300 μL of protein A-Sepharose suspension (Protein A CL-4B in 20 mmol/L borate buffer) was added to 150 μL of serum in conical tubes. Thorough mixing of the protein A suspension is necessary before use. After incubation at room temperature for 15 min with rotation (20 rpm), samples were centrifuged at 2000 g for 5 min at 20 °C. PRL concentrations in the serum (PRL before) and in the supernatant (PRL after) were then immediately assayed using the Elecsys method. To determine the real PRL dilution factor, we assayed seven serum samples without macroprolactin (as shown by gel-filtration chromatography) before and after treatment with protein A. The dilution factor (D) was the ratio PRL before/PRL after. As expected, the dilution factor was close to 3 (mean ± SD, 2.90 ± 0.09), showing the absence of matrix effects from the protein A-Sepharose treatment and from nonspecific binding of PRL to the Sepharose. The percentage of protein A-precipitated PRL was calculated as follows: 100 × (PRL before − PRL after × D)/PRL before. Interassay reproducibility was assessed over a 3-month period through repeated analysis (n = 10) of two patient sera, S1 and S2. CVs were 12% for S1 (protein A-precipitated PRL, 31.2%; PRL before, 2050 mIU/L) and 4.1% for S2 (protein A-precipitated PRL, 74.0%; PRL before, 1580 mIU/L). It has been shown that the percentages of macroprolactin established by protein A precipitation and by gel-filtration chromatography, the reference method to identify macroprolactin, are well correlated (15).

Comparative PEG- and protein A-precipitated PRL results are shown in Fig. 1. In our population of 116 hyperprolactinemic samples, 76 yielded PEG-precipitated PRL values <50% and were considered as negative for macroprolactin. In these 76 sera, the mean precipitated PRL was 31.6% (SD, 6.8%; range, 7–49%) with PEG and 0.4% (SD, 6.8%; range, −13.3% to 13.7%) with protein A. The percentage of PRL precipitated was lower for protein A-Sepharose than with PEG precipitation. In fact, the mean percentage of protein A-precipitated PRL did not differ from zero in these macroprolactin-negative samples.

Thirty-one sera yielded PEG-precipitated PRL values >60% and were considered as positive. In these 31 sera, precipitated PRL results were 76.8% (SD, 8.1%; range, 61–96%) with PEG and 55.1% (SD, 16.3%; range, 24–92%) with protein A. In the positive group, the percentage of protein A-precipitated PRL was clearly increased in all samples, indicating that hIgG-PRL complexes were present in these samples. The percentage of protein A-precipitated PRL was positively correlated with the percentage of PEG-precipitated PRL (r = 0.82). Among the nine samples in the gray zone defined with the PEG precipitation test, four were clearly negative with the protein A test (PEG, 52%, 54%, 58%, and 58%, respectively; protein A, 10%, −1.7%, −0.3%, and 10%, respectively), two samples (PEG-precipitated PRL, 56% and 59%) had markedly increased protein A results (32% and 25%, respectively), and the last three samples (PEG-precipitated PRL, 50%, 50%, and 53%) had protein A results (15%, 18%, and 22%) between the higher value in the negative group (14%) and the lower value in the positive group (24%).

All samples with PEG results >60% yielded a high percentage of protein A-precipitated PRL (>24%), and all samples with PEG results <50% yielded a low percentage of protein A-precipitated PRL (<14%). As expected, the results obtained in the nine samples with PEG-precipitated PRL between 50% and 60% were less clear-cut. Four of the nine samples (there was no serum left available for the other five samples) were subjected to gel-filtration chromatography (15). Three samples with markedly (25% and 32%) or moderately (22%) increased protein A-precipitated PRL contained substantial amounts of high-molecular mass PRL (38%, 37%, and 29%, respectively) as determined by gel-filtration chromatography. The last sample with a low protein A-precipitated PRL result...

![Fig. 1. Percentages of PEG- and protein A-Sepharose-precipitated PRL in 116 hyperprolactinemic serum samples (prolactin >600 mIU/L), as determined with the Elecsys PRL assay. The vertical lines indicate the 50% and 60% percentages after PEG precipitation and define the gray area of the PEG test. Samples with PEG-precipitated PRL >60% are considered positive for macroprolactin, whereas samples with PEG-precipitated PRL <50% are negative.](image-url)
(10%) contained 35% high-molecular mass PRL, and the chromatographic profile of this serum showed a marked heterogeneity of macroprolactin with the presence of molecular forms of mass higher than the 150- to 170-kDa macroprolactin. This sample might contain forms of macroprolactin other than hlgG-PRL complexes, high-molecular mass aggregates of PRL or hlgA, or hlgM-PRL complexes (hlgA and hlgM are only in part bound to protein A) (13). This finding underlines the heterogeneity of macroprolactin.

Similar to precipitation with anti-hlgG-agarose, protein A precipitation is more expensive than the PEG test. Its use therefore could be restricted to the evaluation of borderline samples. Compared with immunoprecipitation with anti-hlgG-agarose resin, protein A-Sepharose precipitation has two advantages: a shorter incubation time and, above all, a much lower dilution factor, allowing macroprolactin to be detected in samples with moderate hyperprolactinemia (600 mIU/L as determined with the Elecsys assay). We have found 154 macroprolactin-precipitated PRL in seven serum samples with PRL concentrations <600 mIU/L as determined with the Elecsys assay. In our 3-year experience with the PEG precipitation test and the Elecsys PRL assay, we have found 154 macroprolactin-positive sera. Among these sera, 51% had a PRL concentration <1000 mIU/L (macroprolactinemia is most frequently associated with moderate hyperprolactinemia), and 15% had a concentration <600 mIU/L. We compared PEG- and protein A-precipitated PRL values in seven sera with PRL concentrations <600 mIU/L and PEG-precipitated PRL ≥50% (Table 1). Four samples were positive with both the PEG and with the protein A precipitation tests, and, as observed previously in hyperprolactinemic sera, for the other three samples in the gray zone of the PEG test, the results of the protein A test were different: one was negative, one was positive, and the third sample was in the borderline zone of the protein A test (14–24%). In our opinion, it can also be useful to detect macroprolactin in a sample with a PRL concentration within reference values, e.g., to discontinue inappropriate anti-PRL therapy.

Contrary to the PEG test, the protein A precipitation test showed negligible nonspecific PRL fixation, as evidenced by the fact that dilution did not alter the result from the expected value. In the PEG-negative group, the mean protein A-precipitated PRL value was near zero. This study confirms that the PEG precipitation method can be used routinely with benefit with the Elecsys PRL assay. High amounts of PRL-hlgG complexes were detected by the protein A-Sepharose test in all samples with a PEG test result >60%. That might not be always the case if macroprolactin is composed of PRL aggregates or of hlgG-, hlgA-, or hlgM-PRL complexes. The protein A test is also reliable and may help resolve cases in the gray zone of the PEG test (3.6% of 1091 sera screened for macroprolactin in our experience). In this case, however, should the protein A test result be negative, as clearly shown by the results obtained for one of our serum samples (PEG-precipitated PRL, 52%; protein A-precipitated PRL, 10%; high-molecular mass PRL by gel-filtration chromatography, 35%), the presence of high-molecular mass forms of PRL should be assessed by gel-filtration chromatography.

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References

Table 1. Percentages of PEG- and protein A-Sepharose-precipitated PRL in seven serum samples with PRL concentrations <600 mIU/L as determined with the Elecsys assay.

<table>
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<tr>
<th>Patient</th>
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<tr>
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<td>314</td>
<td>70</td>
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Release of Macromolecular Cardiac Troponin I Complex after Successful Percutaneous Transluminal Coronary Angioplasty in Acute Myocardial Infarction, Alain Lavoinne,1* Bruno Cauliez,1 Hélène Eltchaninoff,2 Christophe Tron,2 and Alain Cribier2

Cardiac troponin I (cTnI) has been proposed for use in early assessment of reperfusion therapy (1–4). However, commercial cTnI assays may react differently depending on the circulating forms of cTnI (5). Numerous reports have focused on the existence of troponin I complexes with the two other troponin components in the bloodstream of patients with myocardial infarction (6, 7). Independent of cTnI proteolysis occurring in the bloodstream related to the existence of covalent complexes of cTnI with cardiac troponin C (cTnI-cTnC) and T (cTnT-cTnI-cTnC) (8), cTnI degradation products also occur within human myocardium (9). To our knowledge however, no report has identified the cTnI forms released in patients with acute myocardial infarction (AMI) undergoing reperfusion therapy. Our purpose was to study cTnI release in patients undergoing successful (TIMI 3) reperfusion by primary percutaneous transluminal coronary angioplasty (PTCA) with stenting to ascertain and visualize rapid restoration of the coronary flow.

We first established the release characteristics of cTnI and creatine kinase MB (CK-MB) in 11 AMI patients [9 males and 2 females; age range, 51–80 years; 4 with an occluded right coronary artery (RCA), and 7 with an occluded left anterior descending artery (LAD)] undergoing PTCA who presented within 6 h after onset of chest pain (2–6 h). All patients received standardized adjunctive therapy consisting in abciximab, heparin, clopidogrel, and aspirin. Plasma (heparin) cTnI (10) and CK-MB concentrations were measured on the DPC Immulite [upper reference limit (URLs): cTnI, 1 μg/L (ROC curve); CK-MB, 4.8 μg/L (99th percentile)]. Samples were taken before PTCA (0 min) and 30, 60, 90, and 120 min afterward. As shown in Table 1, the cTnI concentration before PTCA was below the URL in all of the studied patients. The release profiles of cTnI and CK-MB linearly increased during this period: y = 1.14x – 2.91 μg/L (r = 0.998) and y = 2.37x + 11.78 μg/L (r = 0.999), respectively.

We attempted to identify cTnI forms (free, complexed, and degraded cTnI) released in four different patients by use of fast protein liquid chromatography (FPLC; Pharmacia) with prepacked Superdex 200 HR 10/30 columns (separation range, 10–600 kDa), but this method does not differentiate phosphorylated or oxidized cTnI forms. Elution was performed in 0.05 mol/L phosphate buffer (pH 7.0) containing 0.15 mol/L NaCl as recommended by the manufacturer (flow rate, 0.4 mL/min; plasma sample, 0.5 mL; fraction volume, 0.5 mL). To avoid any change in cTnI forms after sampling, the fractionation procedure was performed immediately after blood collection. For molecular mass measurement of the cTnI forms, calibration was performed by measuring IgG (160 kDa), CK (85 kDa), and myoglobin (17 kDa) in each elution sample, providing only approximate masses. IgG and myoglobin were measured by immunoassays (BN II; Dade-Behring) and total CK activity was assessed at 37°C according to IFCC recommendations with a LX-20 analyzer (Beckman Coulter).

A typical chromatogram obtained for cTnI (also measured on the DPC Immulite) in a sample obtained 120 min after PTCA (patient A, with RCA; see Table 1) is shown in Fig. 1A, with the corresponding chromatograms for IgG, CK, and myoglobin shown in Fig. 1B. Unexpectedly,