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Macroamylase, Macro Creatine Kinase, and Other Macromolecules

Editors: Theodore E. Mifflin and David E. Bruns
Presenters: Ute Wrotnowski, R. Hunt McMillan, and Robert G. Stallings
Discussants: Robert G. Stallings, Ute Wrotnowski, and Theodore E. Mifflin
Consultants: Robin A. Felder and David A. Herold

The importance of macromolecules has become increasingly apparent in recent years (1, 2). Macroamylase (EC 3.2.1.1) and macro CK (EC 2.7.3.2) are the macromolecules most commonly noted in the clinical laboratory, and they are frequently responsible for diagnostic confusion. Several methods are available for the confirmation and analysis of macromolecules, many of which require expensive equipment or complicated techniques.

In this report we summarize two cases of macro creatine kinase and two cases of macroamylase that illustrate the clinical importance of recognizing these macromolecules. We review the features of these macromolecules, discuss their laboratory evaluation, and describe a simple method that we have used to detect the macromolecules in these (and other) patients at our institution. Finally, we review the literature on other, less commonly observed macromolecules in human blood. We stress (a) the impact of methodology on clinical impressions and (b) the importance of discussing laboratory observations with the patient's physician and communicating them to the patient's medical record in writing.

The Cases

Case 1

A 76-year-old white woman was admitted for evaluation of episodic substernal chest pain of recent onset, associated with paroxysmal nocturnal dyspnea. She was obese, with a 20-year history of hypertension, a 55-pack-per-year smoking history, and a family history of cardiac disease. She had rheumatic heart disease at age 12, and she had suffered a transient ischemic attack one month before this admission.

Physical examination revealed atrial fibrillation and pulmonary edema. Nonspecific ST-T-wave changes were noted on the electrocardiogram.

Values for creatine kinase (3) in serum varied from 283 to 322 U/L (reference interval: 0-110 U/L) during a 35-h period. CK-MB isoenzyme was absent by quantitative immunoinhibition/immunoprecipitation assay (4, 5). Agarose gel electrophoresis (6) demonstrated a prominent CK band migrating slightly cathodal to the MB position, suggestive of macro CK. After further laboratory evaluation of this CK (see below) and discussions with the patient's physician, notations of the presence and importance of macro CK were made in the computer-generated laboratory report and in the Progress Notes in the patient's medical record. The patient's pulmonary edema resolved with medical management, but atrial fibrillation persisted.

Six weeks later the patient was seen by her local physician, who ordered total CK and CK-MB determinations, which were performed at an outside laboratory. The results indicated an increased total CK; CK-MB was more than 50% of the total as measured by an immunoinhibition method that did not distinguish between CK-MB and macro CK. The local physician was unaware of the patient's macro CK and these laboratory results led him to suspect myocardial infarction.

Several days later the patient was readmitted to this hospital after a transient ischemic episode and a generalized tonic-clonic seizure. During hospitalization, multiple quantitative and electrophoretic CK determinations demonstrated a transient increase in CK-MM, which peaked at about 600 U/L and which was superimposed on the previously noted macro CK of about 300 U/L. The increased CK-MM was an expected finding, reflecting skeletal muscle damage incurred during the patient's generalized seizure. The physicians caring for the patient were aware of her macro CK, obviating this source of clinical confusion.

Case 2

A 56-year-old white woman was admitted for thyroid lobectomy. She had multiple risk factors for coronary artery disease including obesity, hypertension, and hypercholesterolemia, and had stable exertional angina of 10 years' duration. Preoperative CK assay was not performed. Activities of lactate dehydrogenase (LD, EC 1.1.1.27) and aspartate aminotransferase (AST, EC 2.6.1.1) in serum were not increased.

The patient underwent an uneventful thyroid lobectomy. The histological diagnosis was nodular hyperplasia. An episode of angina in the immediate postoperative period was relieved by nitroglycerin. Subsequent enzyme determinations revealed normal values for LD with persistently above-normal total CK, ranging from 157 to 210 U/L. No CK-MB was detected by quantitative immunoinhibition/immunoprecipitation assay. CK electrophoresis revealed CK-MM plus a band suggestive of macro CK migrating between the normal CK-MM and CK-MB positions. This finding was
discussed with the patient's physician and recorded in the patient's record. Sixteen months later the patient underwent triple-vessel coronary artery bypass grafting at this institution. Total CK during this hospitalization showed a stable borderline increase at 107 to 121 U/L. No isoenzyme determinations were requested.

Case 3

A 53-year-old white woman was admitted for evaluation of indigestion, nausea, and epigastric pain radiating to the back and right shoulder. She had undergone exploratory laparotomy for pancreatitis 26 years previously; hospital records of that admission were unavailable.

Laboratory values included a serum amylase activity (7) of 375 U/L (reference interval: 15-90 U/L) on the day of admission, with values of 257 and 276 U/L on subsequent days. Values for serum calcium and bilirubin were normal. The amylase/creatinine clearance ratio (8) was 0.1% (reference interval 1.3-4.2%). Results of abdominal sonography, cholecystogram, and upper gastrointestinal series were unremarkable. The patient's abdominal pain resolved after five days in the hospital, and she was discharged.

Five years later the patient was readmitted for an unrelated problem (left subclavian artery occlusion). No abdominal symptoms were present. Serum amylase activity was 356 U/L. Amylase electrophoresis demonstrated a faint band in the salivary isoenzyme position plus a broad "smeared" band cathodal to the pancreatic isoenzyme position. The latter was consistent with macroamylasemia and suggested that the previous diagnosis of acute pancreatitis had been erroneous. The very low amylase/creatinine clearance ratio that had been recorded earlier suggested that macroamylasemia had been the explanation for the patient's hyperamylasemia.

Case 4

A 36-year-old alcoholic white man was admitted to the hospital with a complaint of abdominal pain. The patient had a history of drug abuse, alcoholic gastritis, and pancreatitis.

Physical examination showed marked epigastric tenderness. Endoscopic retrograde cholangiopancreatogram revealed distortion of the distal pancreatic duct, consistent with fibrosis.

Serum amylase was persistently increased, ranging from 125 to 459 U/L. Serum lipase (EC 3.1.1.3) activity (9) was 390 U/L (reference interval: 40-240 U/L). Calcium was transiently decreased to 77 mg/L. The clinical course, history of alcoholism, transient hypocalcemia, and increased serum lipase all strongly supported a diagnosis of acute pancreatitis. However, the amylase/creatinine clearance ratio (8) was 0.18% (reference range: 1.3-4.2%). The explanation for this low ratio was apparent on agarose gel electrophoresis (10), which demonstrated a broad "smeared" band of amylase activity anodal to the point of application, suggestive of macroamylase. (The coexistence of macroamylasemia and acute or chronic pancreatitis has been described in at least 11 patients (7).)

Clinical Features of Macroamylase and Macro CK

Macro CK: In serum, macro CK occurs in two major forms (11). Macro CK Type I is a complex composed of an immunoglobulin (usually IgG or IgA) bound to two CK molecules, usually the CK-BB isoenzyme (11). It is detectable in up to 2% of the population, most commonly affects elderly women, frequently persists for months or years, and statistically is not associated with specific diseases (11). Macro CK Type II is oligomeric mitochondrial creatine kinase, released as a result of tissue necrosis. Typically it is a transient finding and is most frequently found in the serum of patients who are terminally ill or who have widespread tissue destruction (11).

The major clinical importance of macro CK lies in its potential for causing confusing laboratory results, particularly during investigation of possible myocardial infarction. Macro CK is indistinguishable from normal creatine kinase in quantitative total CK assays. Misleading supranormal values may be seen in CK-MB assays that rely on a single-step immunoinhibition of M subunits (12). In such assays, residual CK activity is generally presumed to represent CK-MB. Because macro CK Type I (CK-BB) and macro CK Type II each contribute to residual CK activity in these assays, overestimation of CK-MB may result. CK-MB quantification methods that involve a two-step immunoinhibition/immunoprecipitation procedure (4) are not subject to such interference from macro CK.

Each type of macro CK may be noted by electrophoresis, but electrophoresis alone cannot define the macro CK complex. Macro CK Type I usually migrates between the normal MM and MB proteins in agarose gel. Misidentification may result when it overlaps the MM or MB position. Macro CK Type II typically remains near the point of application, where it may be confused with CK-MM (13).

Macroamylase: Macroamylase also occurs in two major forms. "Naturally occurring" macroamylase is a complex composed of an immunoglobulin (IgG or IgA) bound to an amylase molecule—the salivary or pancreatic isoenzyme, or both (14). This form of macroamylase may persist for months or years, and it may be associated with normal or above-normal amylase concentrations in serum (1, 15). Naturally occurring macroamylase has been estimated to be present in serum of 1.0% of normoamylasic and 2.6% of hyperamylasic individuals (1). Iatrogenic macroamylase is an enzyme-substrate complex of amylase and intravenously infused high-molecular-mass glycoprotein or polysaccharide (e.g., hydroxyethyl starch). This form of macroamylase is transient (1, 16).

The major clinical importance of macroamylase lies in its potential to create confusion during the investigation of possible pancreatitis. Immunoglobulin-bound macroamylase is indistinguishable from normal amylase in quantitative amylase assays. An increased value for serum amylase with a normal one for lipase in serum is consistent with, but not specific for, macroamylasemia. Because the renal clearance of macroamylase is low, decreased amylase in the urine or a decreased amylase:creatinine clearance ratio (<1%) can help identify macroamylase (8).

Immunoglobulin-bound macroamylase usually appears as a smeared band on agarose gel electrophoretograms (17), which may overlap the normal position of the salivary or pancreatic isoenzyme or one of their many deamidated forms (18). Thus, when electrophoresis is used alone, the macroamylase may be difficult to distinguish from a normal amylase isoenzyme (1, 18).

The occurrence of macroamylase and macro CK is probably not age related, even though virtually all of the patients studied so far with these macro-complexes have been of middle age or older. That macro enzymes can also occur in pediatric patients has been reported (19-21) only recently (since 1980). Laboratory methods involving gel permeation chromatography were used in two of these studies (19, 20) to confirm the presence of macroamylase. In the third (21), macro CK was reported in some pediatric patients by use of agarose gel electrophoresis. The identity of the complexing protein in these three studies was not established, however.
Additional investigations are needed to define the chemical nature of macroenzyme complexes in children.

Laboratory Evaluation of Macroe nzymes

Electrophoretic/immunological method for macroamylase and macro CK type I. The ability of antisera to human immunoglobulin to precipitate or bind to serum enzymes has been used as presumptive evidence of an enzyme-immunoglobulin complex (I, 2, 11, 22). The following method is used routinely in our laboratory for presumptive identification and characterization of macroamylase and macro CK.

Add 25 μL of patient's serum to each of three test tubes. Add 100 μL of isotonic saline to the first tube, 100 μL of goat anti-human IgG to the second, and 100 μL of goat anti-human IgA to the third. Vortex-mix, seal, and refrigerate the tubes overnight at 4 °C. Centrifuge all tubes for 15 min at room temperature and 2000 rpm. Separate the isoenzymes in each supernatant fluid by use of agarose gel electrophoresis. If a macroenzyme species containing IgG or IgA is present, incubation with the appropriate goat antiserum will either precipitate the macroenzyme or change its electrophoretic mobility. (If desired, an anti-goat Ig antibody can be added to enhance precipitation.) The presence of an immunoglobulin-bound macro enzyme is confirmed when the aberrant band is changed following treatment with one antiserum and not the other. Any other result is inconclusive.

Immunochemical studies of macro CK, cases 1 and 2. Sera from cases 1 and 2 were analysed by the immunochemical method, outlined above, and CK electrophoresis* (Figure 1). The electropherogram for case 1 showed a CK band slightly cathodal to the normal MB position. This band was unaffected by incubation of the serum with saline (lane 2) or goat anti-human IgG (lane 3). Incubation with goat anti-human IgA (lane 4) resulted in a markedly less intense band (as compared with lane 2) and the appearance of a new band at the point of application. Evidently the goat anti-human IgA had bound to the abnormal CK species and altered its electrophoretic mobility. This suggested that it was a macro CK Type I involving IgA.

Case 2 exhibited a normal CK-MM band as well as an abnormal band between the MM and MB positions. The abnormal band was unaffected by incubation with saline (lane 5) or goat anti-human IgA (lane 7). Incubation with goat anti-human IgG (lane 6) resulted in almost complete disappearance of the band. This indicated that it was a macro CK type I involving IgG.

Immunochemical studies of macroamylase, cases 3 and 4. The amylase electrophoretic plate* from case 4 is shown in Figure 2. The abnormal amylase appeared as a smeared band anodal to the point of application (lane 2). This band was unaffected by incubation with saline (lane 2) or goat anti-human IgG (lane 3). Incubation with goat anti-human IgA (lane 3) condensed the band and shifted its position toward the point of application. This result indicated that

* For CK electrophoresis we used 10 g/L agarose gel in the "Corning ACI" system (Corning Medical, Medfield, MA 02052). CK isoenzymes were made visible by use of a fluorometric method (Corning Medical) with creatine phosphate as substrate and NADH as indicator. Omission of creatine phosphate allows identification of adenylate kinase activity; such reagent is available from several suppliers.

* Amylase isoenzymes were electrophoretically separated on 10 g/L agarose gels in the Corning ACI system and made visible with a suspension of starch dyed with Cibachron FSG-A ("Phadebas"; Pharmacia Diagnostics, Piscataway, NJ 08854). Amylase isoenzyme control material was prepared from salivary and pancreatic isoenzyme purified as previously described (53).

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Fig. 1. CK isoenzyme electrophoresis of sera incubated with various antisera
Lane 1 contained a serum that illustrates the normal electrophoretic positions for (left to right) CK-MM, CK-MB, albumin, and CK-BB. Lanes 2, 3, and 4 contain serum from case 1 incubated with saline, goat anti-human IgG, and goat anti-human IgA, respectively. Lanes 5, 6, and 7 contain identical treated aliquots of serum from case 2

Fig. 2. Electropherogram of amylase isoenzymes on 10 g/L agarose gel
Lane 1 contains purified pancreatic (P) and salivary (S) isoenzymes. Lanes 2, 3, and 4 contain serum from case 4, previously incubated with saline, goat anti-human IgA, and goat anti-human IgG, respectively.

the goat anti-human IgA had bound the abnormal amylase and altered its electrophoretic mobility, suggesting that the abnormal amylase was an immunoglobulin-bound macroamylase involving IgA. A similar pattern was observed in
case 3, except that the macroamylase involved IgG rather than IgA (not shown).

Chromatographic studies of macroenzymes. Demonstration that an enzyme has a greater than normal molecular size (i.e., is a macroenzyme) requires the use of techniques that separate the enzymes on the basis of differences in molecular size, in contrast to those screening techniques such as agarose gel electrophoresis that separate macroenzyme species primarily on the basis of differences in charge. For size separations, conventional and "high-performance" liquid-chromatographic methods involving gel-permeation packings can be used.

Figure 3 illustrates the results of column chromatography of serum from case 4 on Bio-Gel P-100. The amylase in the patient's serum was eluted earlier than that in the control, confirming the presence of macroamylase. A similar elution profile was observed with serum from case 3 (results not shown).

To hasten size separations of macroenzymes, we have developed a high-pressure liquid-chromatographic method. Typical results are shown in Figure 4. The amylase in the patient's serum was eluted earlier than control amylase, confirming the presence of macroamylase. Elution profiles with serum from cases 3 and 4 were similar (results not shown).

For chromatography of macroamylase we used a 1 \times 66 cm column packed with Bio-Gel P-100 (Bio-Rad) and equilibrated overnight at room temperature in pH 7.3 buffer (per liter: 50 mmol of NaH₂PO₄, 50 mmol of NaCl) at a flow rate of 6 mL/h. The column was calibrated with Blue Dextran 2000 (Mₙ 2 \times 10⁶) and cytochrome c₁ (Mₙ 16 000). We applied 250 μL of serum and collected 0.5-mL fractions. Amylase activity was measured in individual fractions at 37 °C in an RA 1000 discrete analyzer, with maltotetraose as substrate (7).

For "high-pressure" liquid chromatography of macroamylase we used a Beckman Altex high-performance liquid chromatograph (Model 342) equipped with a 7.5 mm \times 30 cm size-exclusion column (Altex Spherosil TSK, 10 μm, 3000 SW). The column was equilibrated for 2 h at room temp. with pH 7.3 buffer (see footnote 5) at a flow rate of 1 mL/min. The column effluent was monitored at 280 nm. Serum (200 μL) was injected and fractions were collected at 0.4-min intervals for 25 min after the sample was injected. Total amylase activity was determined as previously described (7), except that sample volume was increased from 30 μL to 60 μL.

Fig. 4. Liquid-chromatographic separation of macroamylase on 10-μm Fractogel TSK support
Total amylase activity was 720 U/L in the saliva amylase solution (in 10 g/L bovine serum albumin (Δ-Δ) and 1820 U/L in the serum that contained the macroamylase (●-●).

The results of these studies were conveyed to the patients' records in an attempt to avoid unnecessary future hospitalizations or evaluations.

Other Macroenzymes

Lactate dehydrogenase (LD). Following amylase and CK, LD is the enzyme most frequently reported in a macromolecular form (I). These circulating molecular species result from complex formation involving (a) one or more LD isoenzymes and one of the immunoglobulins (IgA, IgG, or IgM), (b) LD and betalipoprotein (23), or (c) self-association of the individual isoenzymes (24). The immunoglobulin most commonly found (60%) is IgA (I), whereas the complex of IgM and LD is apparently quite rare, having only recently been observed (25). The reasons for the formation of the complexes are unknown, although an autoimmune process has been associated with the immunoglobulin (Ig) type of complex (26, 27).

Macro LD may be manifested in LD isoenzyme electrophoresis as (a) an abnormal number of LD isoenzyme bands, (b) altered electrophoretic mobility of LD isoenzymes, or (c) broadening of existing bands (26). For confirmation of the macro LD species, gel filtration of serum samples on Sephadex G-200 has been used to demonstrate the presence of LD species of high molecular mass (I). Persistent unexplained increases in serum LD may suggest the presence of a macro species, as shown by Klonoff (I), who reviewed 45 cases of macro LD and found increased total serum LD in 85%. In at least one reported case, a macro LD persisted after total LD activity had declined to its reference interval (23).

Alkaline phosphatase (ALP, EC 3.1.3.1). Macro complexes of alkaline phosphatase can be considered in two general categories, depending upon the composition of the complex. In the first category, macrocomplexes of ALP containing any one of the three major immunoglobulin classes (IgA, IgG, IgM) have been described (2, 29, 30), the IgG type being the most commonly observed. The bound ALP isoenzyme has been identified in various reports as hepatic, eosinous, or intestinal in origin. Typically, these complexes migrate in electrophoretic medium with an abnormally slow mobility, obscuring their true identity. Immuno-electrophoresis with anti-serum to human immunoglobulin has proven useful in identifying the immunoglobulin contained within several different ALP macro complexes (29, 30). A weak correlation has been described between the presence of an ALP-IgA macrocomplex and ulcerative colitis (31).
The second type of macro ALP is frequently overlooked in reviews of macroenzymes. It is characterized by extremely high relative molecular mass (Mr 1,000,000) (32) and has been termed “particulate” ALP (33, 34). This form is frequently observed in sera from patients with liver disease, and ALP of similar size has been found in normal bile (35). The high-Mr ALP in serum or bile resembles the low-Mr ALP of liver in both kinetic and inhibition properties (36). The complexing protein is apparently not an immunoglobulin, but is instead lipoprotein-X (33). The incidence of particulate ALP in serum of patients with various hepatic diseases has been examined (37).

Gamma-glutamyltransferase (γ-GT), leucine aminopeptidase (LAP) and 5′-nucleotidase. That γ-GT can exist as a high-molecular-mass form has been known for some time (38-40), but these high-Mr γ-GT species have been characterized only recently with regard to their individual sizes and compositions (32, 41-43). Other macro complexes containing either LAP or 5′-nucleotidase were studied concurrently (33, 43), but less is known about them.

Extensive studies of the macro γ-GT and LAP species reveals a heterogeneous composition which varies according to their molecular size (41-43). The high-Mr, (1,000,000) γ-GT and LAP complexes contain lipoprotein-X, while HDL is incorporated within the intermediate-Mr, (250,000-500,000) γ-GT and LAP species. A low-Mr, (120,000) form of γ-GT is a hydrophilic species, thought to be the product of endopeptidase action upon the hydrophobic enzyme (39). Additional studies have shown that γ-GT can be complexed with apolipoprotein A or B as well as IgA (44, 45).

The question of transport for these “hepatic” macroenzymes (ALP, γ-GT, LAP) remains an object of speculation, because these species can normally be found in both serum and bile (32, 43). The mechanism of their formation also remains unclear, because evidence exists which supports both a membrane-fragment hypothesis (33, 34) and soluble self-aggregation (32). Finally, the diagnostic potential of macro γ-GT has recently been explored (43, 45).

Aminotransferases (AST and ALT). Macrocomplexes have been described (1, 2) involving the immunoglobulins and the aminotransferases AST and ALT (EC 2.6.1.2). Reports describing complexes containing AST are more numerous (six of them since 1978) than those for ALT (two since 1978) (2). IgG appears to be the immunoglobulin usually involved; the cytoplasmic form of AST is the more frequently bound AST isoenzyme. A recent case, however, described macro complexes involving both the cytoplasmic and mitochondrial isoforms of AST in the same patient (46). Using purified isoforms of AST, Nagamine and Okochi (46) determined that the patient’s IgG bound only cytoplasmic AST, whereas the patient’s IGA bound both cytoplasmic AST and mitochondrial AST. Counterimmunoelectrophoresis followed by in-situ staining for AST activity was used to detect enzyme activity within the immunoprecipitin bands.

Other enzymes. For several enzymes frequently measured in serum, we are aware of no evidence of macromolecular complexes. This group includes acid phosphatase (EC 3.1.3.2), aldolase (EC 4.1.2.13), cholinesterase (EC 3.1.1.8), isocitrate dehydrogenase (EC 1.1.1.41), and lipase. In contrast, an enzyme usually studied in erythrocytes, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), reportedly forms a macroenzyme complex of unknown identity (47). Further characterization of this macro enzyme will be necessary to determine its possible clinical significance.

Summary

The four cases described above amply illustrate the potential for diagnostic confusion associated with macroenzymes. Each of the first two patients presented with signs and symptoms suggestive of myocardial infarction. The above-normal total CK activities heightened this suspicion. A one-step immunoinhibition method for CK-MB quantitation produced misleading results. In contrast, a two-step immunoinhibition/immunoprecipitation method demonstrated that the increased CK activity was not due to CK-MB. Similarly, patients 3 and 4 presented with signs and symptoms suggestive of pancreatitis. In each patient an increased serum amylase was present. The finding of macroamylasemia prevented a misdiagnosis of acute pancreatitis in one patient and explained a low amylase clearance in the other patient in whom all other results supported the diagnosis of acute pancreatitis. Two of the cases clearly illustrate the importance of communicating the laboratory’s findings to the medical record in writing.

Various methods have been used to detect macro enzymes (1, 11, 13, 22). Unfortunately, many of these methods involve such complicated or cumbersome procedures as size-exclusion chromatography (20, 25, 36, 38, 42, 43, 48), gradient gel electrophoresis (29, 32, 43), radioelectrophoresis (13), ultracentrifugation (39), thermal stability measurement (49), or activation energy determination (50). A simple method (51) involving precipitation with polyethylene glycol has been evaluated (52) and found useful for detection of macroamylases. The electrophoretic/immunological detection method that we have used for macro CK Type I and macroamylase provides a simple and inexpensive means of indicating the presence of these common macroenzymes and identifying the immunoglobulin class involved (1, 2, 11, 22). This approach has proven useful for the characterization of other immunoglobulin-bound macroenzymes in the clinical laboratory (27).

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References

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