Zašto treba prepoznati makroenzime?

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Zašto treba prepoznati makroenzime?
Why is it necessary to recognize macroenzymes?

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Sažetak

Makroenzimi su kompleksi normalnih enzima ili izoenzima nastalih vlastitom polimerizacijom, najčešće povezivanjem s imunoglobulinima, zatim nekim lipoproteinima, proteinima ili fragmentima staničnih membrana. U pravilu su veće molekularne mase i dužeg poluživota u sistemskoj cirkulaciji. Pojava makroenzima u serumu može se odraziti na analitički proces mjerenja aktivnosti enzima te biti uzrokom pogrešne interpretacije povećane aktivnosti enzima zbog slabog razumijevanja kliničkog značenja prisutnosti makroenzima.

Ključne riječi: makroenzimi, makroCK, imunoglobulini

Summary

Macroenzymes are complexes consisting of normal enzymes or isoenzymes generated by their polymerization, mostly by their linking to immunoglobulins, some lipoproteins, proteins, or cell membrane fragments. Generally, macroenzymes are characterized by a greater molecular mass and longer half-life in systemic circulation. The occurrence of macroenzymes in serum may influence analytical measurement of an enzyme activity or may cause erroneous interpretation of elevated enzyme activity due to inadequate understanding of the clinical relevance of the presence of macroenzymes.

Key words: macroenzymes, macro creatine kinase, immunoglobulins

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Uvod

Jedan od relativno rijetkih uzroka visoke aktivnosti enzima je i prisutnost različitih makroenzima u serumu. U znanstveno-stručnoj literaturi su opisani brojni makroenzimi (1-4), a najbolje pojava makroenzima amilaze, otkrivenog prvi puta 1964. godine (5). Makroenzimi su normalni enzimi (ili izoenzimi) u serumu koji stvaraju komplekse visoke molekularne mase vlastitom polimerizacijom, povezivanjem s drugim sastojcima seruma veće molekularne mase, najčešće imunoglobulinima, ili su kompleksi s dijelovima staničnih membrana (1). Budući da imaju veliku molekularnu masu, makroenzimi imaju sporiji klirens te se nakupljaju u serumu i tako povećavaju aktivnost odgovarajućeg enzima. Ovi se oblici enzima u pravilu otkrivaju kod bolesnika koji imaju neobjašnjeno stalno povećanu aktivnost enzima u serumu, koja se ne uklapa u opću kliničku sliku. Zbog toga mogu interferirati kod interpre-

Introduction

The presence of various macroenzymes in serum is one of the relatively rare causes of high enzyme activity. Numerous macroenzymes have been described in the literature (1-4), of which the occurrence of the macroenzyme amylase, first discovered in 1964 (5), is best characterized. Macroenzymes are normal serum enzymes (or isoenzymes), which form high molecular mass complexes by their polymerization, linking with other serum high molecular mass constituents, mostly immunoglobulins, or complex with cell membrane segments (1). Macroenzymes undergo a slower clearance rate due to their high molecular mass, thus accumulating in serum and enhancing the respective enzyme activity. These enzyme forms are as a rule detected in patients with continuously elevated serum enzyme activity that cannot be explained and is inconsistent with the general clinical picture. Therefore, macroenzymes

tacije nalaza aktivnosti enzima u serumu, odnosno biti uzrokom dijagnostičkih i terapijskih pogrešaka. Naime, nemogućnost otkrivanja makroenzima kao uzroka neobjašnjenog povećanja aktivnosti enzima u serumu može rezultirati primjenom skupih, nepotrebnih i moguće invanzivnih postupaka u postavljanju alternativne dijagnoze. Weidner i sur. (6) su, primjerice, opisali bolesnicu od 54 godine koja je u više navrata hospitalizirana zbog izolirane visoke aktivnosti aspartat-aminotransfeaze (AST), načinjeno je nekoliko biopsija jetre, konzultacije s neurolozima, endokrinolozima i gastroenterolozima, dok visoka aktivnost nije konačno pripisana prisutnosti makroenzima. Uočeno je da je pojava makroenzimskih oblika nekih enzima povezana s nekim autoimunim poremećajima kao što su reumatoidni artritis, imunodeficijencija imunoglobulina A, sistemski eritematozni lupus i ankilozni spondilitis (7-9), te s nekim malignim bolestima, primjerice karcinomom želuca, dojke, prostate te slučajnim stanjima (10,11). Tako su, npr., pregledavanjem medicinskih zapisa između 1988. i 1990. godine na Klinici Mayo kod 42 bolesnika nađeni različiti makroenzimi (4). Dijagnoze koje su zabilježene kod bolesnika s otkrivenim makroenzimima prikazane su u tablici 1., a zamijećeno je da su bolesnici s makro kreatin-kinazom (makroCK) i makro laktat-dehidrogenazom (makroLD) bili stariji od 60 godina, dok su oni s pojavom makroAST bili mlađi.

Smatra se općenito da u korist prisutnosti makroenzima govori a) odsutnost simptoma uz navedenu visoku aktivnost enzima, b) prisutnost simptoma atipičnih za visoku aktivnost enzima te c) izolirana, stalno povećana aktivnost enzima, bez vremenske dinamike. Trajanje prisutnosti makroenzima u serumu opisano u literaturi je u od nekoliko dana, mjeseci, nekoliko godina do trajne prisutnosti.

may interfere with interpretation of the serum enzyme activity findings and thus lead to diagnostic and therapeutic errors. A failure to detect macroenzymes as the cause of unexplained elevation in the serum enzyme activity may result in the use of expensive, unnecessary and possibly invasive procedures in making an alternative diagnosis. For example, Weidner et al. (6) describe a 54-year-old female patient hospitalized on several occasions for isolated high activity of aspartate aminotransferase (AST). She had undergone repeat liver biopsies and consultations with neurologists, endocrinologists and gastroenterologists before the high enzyme activity was eventually ascribed to the presence of macroenzyme. The occurrence of the macroenzyme forms of some enzymes has been associated with certain autoimmune disorders such as rheumatoid arthritis, IgA immunodeficiency, systemic lupus erythematosus (SLE) and ankylosing spondylitis (7-9), with some malignant diseases such as carcinoma of the stomach, breast and prostate, as well as some accidental conditions (10,11). A survey of medical records between 1988 and 1990 at Mayo Clinic revealed the presence of various macroenzymes in 42 patients (4). The diagnoses recorded in patients with detected macroenzymes are listed in Table 1. It should be noted that patients with macro creatine kinase (macroCK) and macro lactate dehydrogenase (macroLD) were older than 60, whereas those with macroAST were younger.

Generally, the presence of macroenzymes is considered to be suggested by (a) the absence of symptoms in association with high enzyme activity; (b) the presence of symptoms atypical for high enzyme activity; and (c) isolated, continuously elevated enzyme activity showing no variation with time. According to literature data, the

Tablica 1. Dijagnoze 42 bolesnika s prisutnošću makroenzima (4)

Table 1. Diagnoses in 42 patients with presence of macroenzymes (4)

Disease type	Specific conditions
Autoimmune	Thyroiditis, rheumatoid arthritis, Sjögren's syndrome
Cardiovascular	Myocardial infarction, hypertension, cardiac arrest, congestive heart failure, atrial fibrillation, peripheral vascular disease, etc.
Endocrine	Diabetes mellitus, hypothyroidism, thyroiditis, multinodular goiter
Gastrointestinal	Chronic pancreatitis, hepatitis, small intestinal obstruction, Crohn's disease, pancreatic carcinoma, etc.
Hematologic	Anemias, lymphomas
Infectious	Pyelonephritis, mastitis, bacteremia, pneumonia, emphysema, etc
Malignant	Carcinoma of the liver, pancreas, uterus, breast, urinary bladder, lungs, prostate, etc.
Pulmonary	Chronic obstructive pulmonary disease, bronchiectasias, pneumonia, emphysema
Renal	Chronic and acute renal failure, pyelonephritis
Rheumatologic	Gout, pseudogout, osteoarthritis, rheumatoid arthritis, Sjögren's syndrome

U otkrivanju i definiranju ovih kompleksa obično se primjenjuju tehnike kao što su elektroforeza, imunoinhibicija, imunoprecipitacija i kromatografija, a vrlo često potrebno je primijeniti i kombinaciju različitih tehnika.
Makroenzimi se obično klasificiraju u dvije temeljne skupine: makroenzime tipa 1 (tablica 2.) i makroenzime tipa 2 (tablica 3.).

length of the macroenzyme presence in serum may vary from days, months and years through permanent presence. Techniques such as electrophoresis, immunoinhibition, immunoprecipitation and chromatography are usually employed to detect and define these complexes. Quite frequently, a combination of techniques has to be used.
Macroenzymes are generally classified into two main groups: macroenzymes type 1 (Table 2) and macroenzymes type 2 (Table 3).

Tablica 2. Makroenzimi tipa 1 izolirani iz seruma bolesnika (12)

Table 2. Macroenzymes type 1 isolated in patient sera (12)

Macroenzyme	Diagnosis	Ig type	Ig specificity
ALT	Chronic hepatopathies	IgG	No data
ALP	Various diagnoses	IgG, IgA	Isoenzymatic specificity
Amylase	Various diagnoses	IgG, IgA	Rare isoenzymatic specificity
AST	Healthy subjects, various diagnoses	IgG (IgA)	Partial isoenzymatic specificity
GGT	Hepatobiliary diseases	IgA	No data
CK	Healthy subjects, various diagnoses	IgG, IgA	BB specificity
LD	Healthy subjects, various diagnoses	IgA, IgG	Mostly H or M specificity
Lipase	Hodgkin disease	IgG	Lipase specificity
ACP	Various diagnoses	IgG	No data

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; CK, creatine kinase; LD, lactate dehydrogenase; ACP, acid phosphatase; Ig, immunoglobulin

Tablica 3. Makroenzimi tipa 2 izolirani iz seruma bolesnika (12)

Table 3. Macroenzymes type 2 isolated in patient sera (12)

Macroenzyme	Diagnosis	Mechanism of formation
ALP	Hepatobiliary disease	Binding to lipoproteins
Amylase	Iatrogenic disease	Binding to drugs
GGT	Hepatobiliary disease	Binding to lipoproteins
CK	Hepatic disease, malignancy	Polymerization
LAP	Hepatobiliary disease	Binding to lipoproteins

ALP, alkaline phosphatase; GGT, γ-glutamyltransferase; CK, creatine kinase; LAP, leucine aminopeptidase

Makroenzimi tipa 1

Pod ovim tipom makroenzima podrazumijevaju se makroenzimi nastali povezivanjem enzima (češće pojedinih izoenzima) u serumu sa specifičnim imunoglobulinima,

Macroenzymes type 1

This type includes the macroenzymes formed by serum enzyme (or more frequently particular isoenzyme) linking with specific immunoglobulins, mostly IgG and IgA cla-

najčešće IgG i IgA klase, a rjeđe IgM klase. Još uvijek nije jasan razlog stvaranja kompleksa enzim-imunoglobulin. Premda se neki proteini vežu na imunoglobuline nespecifično, svi relevantni analitički postupci istraživanja pokazuju da kompleks enzim-imunoglobulin ima značajke kompleksa Ag-At (1,2). Opisani odnosi imunoglobulin:enzim (izoenzim) u kompleksima kreću se uglavnom u odnosima od 2:1, 1:1 ili 1:2, pa su sukladno tome i različite molekularne mase makroenzima. Specifično mjesto vezanja enzima je najčešće na Fab i $F(ab^1)_2$ dijelu imunoglobulinske molekule (13,14). Vezanje enzima na Fab fragment imunoglobulina stabilizira enzimsku aktivnost protiv visoke temperature, smanjuje razinu eliminacije i utječe na kinetičke parametre enzima. U većini slučajeva vezanje enzima na imunoglobulin nema učinka ili ima tek slab inhibicijski učinak na aktivnost pojedninog enzima (12). Nadalje, prema dosadašnjim saznanjima čini se da je stvaranje kompleksa uzrokovano abnormalnostima strukture imunoglobulina, a ne molekule enzima (2). U nastavku teksta sažeto se navode značajke nekih makroenzima ove skupine:

a) **Makroenzim alkalne fosfataze tipa 1** (**makroALP)**, otkriven je prvi puta 1975. godine (15), a procjenjue se da mu je incidencija 0,3-0,4% u serumima u kojima se određuju izoenzimi ALP. Izoenzim u kompleksu je najčešće koštani ili jetreni oblik, a imunoglobulinska klasa koja je češće zastupljena je IgG, obično *λ* podtip te rjeđe *κ* podtip. Opisane molekularne mase makroenzima kreću se u rasponu od 280 000 do 540 000, ovisno o bolesniku. Aktivnost ALP kod bolesnika s makroALP ovog tipa obično je povećana dvostruko, ali su opisane i veće kao i normalne aktivnosti. Povećana aktivnost traje nekoliko mjeseci ili godina. Kao za većinu makroenzima nije jasna povezanost s bolestima, ali prema nekim radovima češće su to poremećaji autimunog podrijetla.

b) **Makroenzim amilaze tipa 1 (makroamilaza)**, kompleks od jedne molekule imunoglobulina i jedne molekule amilaze pokazuje učestalost od 1,0% u populaciji s normalnom aktivnošću amilaze i 2,5% kod bolesnika s visokom aktivnošću amilaze (16), ali ima podataka i o većoj učestalosti (17). Prema objavljenim podacima, imunoglobulinski dio kompleksa uključuje IgA*κ*, IgA*λ*, IgG*κ*, ili IgG*λ*. Karakteristična slika kod bolesnika s makroamilazom u serumu je 1,5-8 puta povećana aktivnost enzima u serumu i nenormalno nizak odnos klirens amilaza:kreatinin. Opisana su i dva slučaja istodobne pojave makroamilaze i makrolipaze u serumu bolesnika s glutenskom enteropatijom (17) i sistemskim eritematoznim lupusom (18).

c) **Makroenzim aspartat-aminotransferaze (makroAST)** prvi je puta opisan 1978. godine (19), a kasnije je ovaj makroenzim opisan u dvadesetak bolesnika s različitim dijagnozama. Najčešće su to akutni i kronični he-

ss, and less frequently IgM class. The reason for the formation of the enzyme-immunoglobulin complexes remains unknown. Although some proteins undergo nonspecific linking to immunoglobulins, all relevant analytical procedures indicate the enzyme-immunoglobulin complex to have the characteristics of Ag-At complex (1,2). The 2:1, 1:1 or 1:2 immunoglobulin to enzyme (isoenzyme) ratios have mostly been described in these complexes, resulting in the respective macroenzyme molecular mass variation. The specific enzyme binding site is mostly on the Fab and $F(ab^1)_2$ fragment of the immunoglobulin molecule (13,14). The enzyme linking to the immunoglobulin Fab fragment stabilizes enzyme activity against high temperature, reduces the level of elimination, and influences kinetic parameters of the enzyme. In most cases, the enzyme linking to immunoglobulin has no or only a weak inhibitory effect on the particular enzyme activity (12). According to current concepts, the formation of complexes appears to be induced by structural immunoglobulin rather than enzyme molecule abnormalities (2).

Characteristics of some of this group macroenzymes are briefly presented below.

a) **The alkaline phosphatase macroenzyme type 1 (macroALP)** was first discovered in 1975 (15). Its prevalence has been estimated to 0.3%-0.4% of sera in which ALP isoenzymes are being determined. The bone or hepatic form of isoenzyme is mostly present in the complex, and is predominated with the IgG immunoglobulin class, usually λ subtype, and less commonly κ subtype. The macroenzyme molecular mass has been reported to range from 280 000 to 540 000, and is patient dependent. In patients with this type of macroALP, the activity of ALP usually shows a twofold increase, however, higher as well as normal activities have also been reported. The increased enzyme activity may persist for months or years. Like the majority of macroenzymes, the association of macroALP with particular diseases remains unclear, however, some studies suggest it to be more frequently associated with autoimmune disorders.

b) **The amylase macroenzyme type 1 (macroamylase)**, a complex formed of one immunoglobulin molecule and one amylase molecule, shows a prevalence of 1.0% in the population with normal amylase activity and of 2.5% in patients with high amylase activity (16); however, a higher prevalence has also been reported (17). According to literature data, the immunoglobulin part of the complex includes IgAκ, IgAλ, IgGκ or IgGλ. The picture in patients with macroamylase present in serum is characterized by 1.5- to 8-fold serum enzyme activity and abnormally low amylase:creatinine clearance. Two cases of simultaneous occurrence of macroamylase and macrolipase in serum of patients with gluten enteropathy (17) and SLE (18) have been described.

patitis, maligne i autoimune bolesti, ali nađen je i kod zdravih osoba (20,21). Imunoglobulinska komponenta je u većini slučajeva bio IgG te rjeđe IgA. Prevalencija u općoj populaciji nije poznata, ali se smatra kako procjenu prisutnosti makroAST treba uvijek načiniti kod asimptomatskog povećanja aktivnosti AST (16).

d) **Makroenzim laktat-dehidrogenaze tipa 1 (makroLD)**, češće se pojavljuje u nekim bolestima kao što su neoplazme, bolesti jetre, kardiovaskularni poremećaji (22-24), u nekoliko slučajeva bolesnika s opekotinama (22), ali je nađen i kod zdravih osoba (25). U jednom od objavljenih radova na većem broju bolesnika opisana je prevalencija od 0,03% te prosječno trostruko povećanje aktivnosti LD kod ispitanika s otkrivenom makroLD. Kao imunoglobulinske komponente najčešće se pojavljuju IgG i IgA, a opisano je i nekoliko kompleksa s IgM te istodobno IgG i IgM, kao i jedan slučaj s IgG i IgA, uz različit udio lakih lanaca lambda i kapa. Molekularne mase makroenzima kreću se najvećim dijelom od 420 000 do 490 000, ali su opisani i kompleksi s Mr > 600 000 i < 1 000 000. Još uvijek nije jasno odgovara li povezivanje LD s imunoglobulinom specifičnoj reakciji Ag-At, budući da ima dosta podataka i o nespecifičnim interakcijama (26).

e) Tipična narav **makroenzima kreatin-kinaze tipa 1 (makroCK)**, je kompleks izoenzima CK-BB i IgG, ali su mogući i kompleksi drugih izoenzima i imunoglobulina (4). Jedan od objavljenih radova navodi prevalenciju makroCK ovoga tipa od 0,43-1,2% (27). Međutim, prevalencija ovisi o metodi određivanja, dobi, spolu i značajkama bolesti ispitanika. Ukupna aktivnost može biti povećana od 3 do 18 puta, ali može biti i normalna. U pravilu odnos aktivnosti CK-2:ukupna CK > 0,25 upućuje na prisustnost makro-CK. Kada se načini elektroforeza na celuloza acetatnim trakama makro-CK tipa 1 lokalizira se između CK-MM i CK-MB (makro CK tipa 2 se lokalizira više katodno u usporedbi s CK-MM). Klinička značajnost makroCK tipa 1 nije jasno određena, ali se većim dijelom povezuje s autoimunim poremećajima. Kako pojava makroenzima može izazvati zabunu u interpretaciji nalaza bolesnika, u svome radu na primjeru makroCK opisuju Galarraga i sur. (28).

Makroenzimi tipa 2

Iz skupine makroenzima tipa 2 u literaturi je najviše podataka o makroCK tipu 2 te manji broj podataka o ALP, GGT te amilazi, lipazi, 5-nukleotidazi i leucin-aminopeptidazi (LAP). Ovaj tip makroenzima definira se kao skupina makroenzima nevezanih za imunoglobuline. Radi se zapravo o kompleksima enzima nastalih vlastitom polimerizacijom (29), povezivanjem s drugim sastojcima seruma, primjerice hidroksietil škrobom (30), lipoproteinima kao što su

c) **The aspartate aminotransferase macroenzyme (macroAST)** was first described in 1978 (19). Later on, this macroenzyme has been reported in some 20 patients with various diagnoses, most frequently acute and chronic hepatitis, malignant and autoimmune diseases, however, it was also found in healthy subjects (20,21). In most cases, the immunoglobulin component was IgG, less frequently IgA. The prevalence of macroAST in the general population is unknown. It is considered that the presence of macroAST should always be tested in case of asymptomatic elevation of AST activity (16).

d) **The lactate dehydrogenase macroenzyme LD type 1 (macroLD)** is more frequently present in some diseases such as neoplasms, liver disease and cardiovascular disease (22-24), however, it has also been reported in some burn patients (22) as well as in healthy subjects (25). In a study performed in a large number of patients, a 0.03% prevalence of macroLD and a threefold increase in LD activity on an average were found in subjects with macroLD detected in serum. The most common immunoglobulin components are IgG and IgA; a number of complexes with IgM, with IgG and IgM concurrence, and one case with IgG and IgA concurrence have also been described, with a varying prevalence of lambda and kappa light chains. The macroenzyme molecular mass mostly ranges between 420 000 and 490 000, however, complexes with Mr > 600 000 and < 1 000 000 have been reported. It has not yet been clarified whether LD linking to immunoglobulin corresponds to a specific Ag-At reaction, as there also are data on nonspecific interactions (26).

e) The isoenzyme CK-BB and IgG complex typically characterizes the **creatine kinase type 1 macroenzyme (macroCK)**, although complexes formed of other isoenzymes and immunoglobulins may also occur (4). The reported prevalence of this macroCK type is 0.43%–1.2% (27). However, its prevalence depends on the method of determination, age, sex and disease characteristics. Total enzyme activity may rise 3- to 18-fold, or may be normal. The CK-2 to total CK activity ratio > 0.25 as a rule points to the presence of macroCK. On cellulose acetate electrophoresis, macroCK type 1 is localized in-between CK-MM and CK-MB (macroCK type 2 is localized more cathodically in comparison with CK-MM). The clinical relevance of macroCK type 1 has not yet been fully clarified, however, it has mostly been associated with autoimmune disorders. In their study, Galarraga *et al*. show, taking the example of macroCK, how the occurrence of macroenzymes may confound the interpretation of patient findings (28).

Macroenzymes type 2

In the group of macroenzymes type 2, most literature data refer to macroCK type 2, and to a lesser extent to ALP,

VLDL, LDL, lipoprotein-X (1), alfa$_2$-makroglobulinom (31) te fragmentima staničnih membrana, što je važno u slučaju tzv. membranskih enzima kao što su ALP, GGT, LAP i 5-nukleotidaza (3,32). Potonji tip makroenzima općenito se javlja kod bolesnika s hepatobilijarnim bolestima, pri čemu se značajna uloga u nastajanju makroenzima pripisuje žučnim solima.

Osim makroCK tipa 2 koji može biti uzrokom lažno pozitivne dijagnoze infarkta miokarda, ovaj tip makroenzima rijetko uzrokuje poteškoće u interpretaciji rezultata u kliničkoj enzimologiji (1,33). Kako s poboljšanjem statusa bolesnika uslijed primijenjene terapije ovi oblici nestaju iz cirkulacije, dakle, prolazne su naravi, smatra se da odražavaju aktivnost ili dinamiku bolesti. U tom smislu, smatra se da bi ovaj tip makroenzima mogao biti klinički zanimljiv zbog potencijalne uloge biljega bolesti.

Hepatobilijarni makroenzimi mogu biti korisni u razlikovanju ekstrahepatične od intrahepatične opstrukcije, za što trenutno ne postoji drugi zadovoljavajući laboratorijski postupak. Još 1985. godine Wenham i sur. su ustanovili osjetljivost od 88% i specifičnost od 96% u razlikovanju ektrahepatične i intrahepatične opstrukcije mjerenjem aktivnosti kompleksiranog oblika GGT (34). Turecky i sur. smatraju, nadalje, da procjena aktivnosti GGT povezane s VLDL+LDL lipoproteinima može biti koristan doprinos u razlikovanju kroničnih hepatopatija od malignih bolesti jetre (35). U razlikovanju kroničnog aktivnog hepatitisa i ciroze jetre pokazana je dijagnostička osjetljivost od 87% i specifičnost od 65%.

MakroCK tipa 2

Opisana prevalencija makroCK tipa 2 je 0,5–3,7%. Smatra se da je ovaj tip makroenzima podrijetlom iz mitohondrija (oligomerna mitohondrijska CK), a njegova pojava u serumu većinom se povezuje s malignim promjenama (3x veća incidencija malignih bolesti nego kod pojave makroCK tipa 1) i s bolestima jetre (29,36).

Wright i Liggett (27) čak preporučuju da se kod bolesnika s povećanom CK ili CK-MB, bez dokaza da se radi o srčanom ili mišićnom poremećaju, načine neinvazivne pretrage za isključenje maligne bolesti: analiza mokraće, okultno krvarenje u stolici, PSA, CA-125, CEA, mamografija, radiološki pregled pluća i UZV zdjelice. Pojava makroenzima povezana je također s većom smrtnošću (29,36), a kada je makroCK tipa 2 prisutan u serumu djece, onda predstavlja biljeg srčane bolesti. Jedan od pretpostavljenih mehanizama povezanosti makroCK tipa 2 s malignim bolestima je izravno otpuštanje enzima iz malignih ili nekrotičnih stanica (29). Pojava makroenzima nije obvezno povezana s povećanjem ukupne aktivnosti CK, ali nekim imunoinhibicijskim postupcima određivanja CK-MB moguća je interferencija makroCK (2). Međutim, važno je napomenuti da makroenzime CK treba razmatrati kod bole-

γ-glutamyltransferase (GGT), amylase, lipase, 5-nucleotidase and leucin aminopeptidase. This type is defined as a group of macroenzymes unbound to immunoglobulins. These are enzyme complexes formed by their polymerization (29) and linking to other serum components, e.g., hydroxyethyl starch (30), lipoproteins such as VLDL, LDL, lipoprotein-X (1), α_2-macroglobulin (31), and cell membrane fragments, which is highly relevant in case of so-called membrane enzymes such as ALP, GGT, leucin aminopeptidase, LAP and 5-nucleotidase (3,32). The latter type of macroenzymes generally occurs in patients with hepatobiliary diseases, where bile acids play a major role in the formation of macroenzymes.

Besides macroCK type 2, which may lead to a false-positive diagnosis of myocardial infarction, this type of macroenzymes rarely causes difficulties in the interpretation of results in clinical enzymology (1,33). As these forms disappear from the circulation with improvement in the patient's condition following appropriate therapy, indicating them to be of transient nature, they are believed to reflect the activity or dynamics of the disease. Therefore, this macroenzyme type may prove to be of clinical relevance for its potential role of a disease marker.

Hepatobiliary macroenzymes may be useful in differentiating extrahepatic from intrahepatic obstruction, for which there is no other satisfactory laboratory procedure available. As early as 1985, Wenham *et al*. found the complex GGT form activity to have a sensitivity of 88% and specificity of 96% in differentiating extrahepatic and intrahepatic obstruction (34). Furthermore, Turecky *et al*. believe that the assessment of GGT activity associated with VLDL+LDL lipoproteins may contribute considerably to the differentiation of chronic hepatopathies and malignant liver disease (35). A diagnostic sensitivity of 87% and specificity of 65% was demonstrated in differentiating chronic active hepatitis and liver cirrhosis.

MacroCK type 2

The prevalence of macroCK type 2 is reported to be 0.5%-3.7%. This macroenzyme type is considered to originate from mitochondria (oligomeric mitochondrial CK), and its occurrence in serum is generally associated with malignant lesions (in malignant disease, it shows a threefold incidence recorded for macroCK type 1) and liver disease (29,36). The more so, Wright and Liggett (27) recommend that noninvasive testing to rule out malignant disease, e.g., urinalysis, occult bleeding in stool, PSA, CA-125, CEA, mammography, radiological lung examination and pelvis ultrasonography be done in patients with elevated CK or CK-MB without evidence for cardiac or muscular disease. The occurrence of this macroenzyme has also been associated with a higher mortality rate (29,36). When present in children's sera, macroCK type 2 is a marker of heart disease. One of the postulated mechanisms of mac-

snika s koncentracijama CK-MB koje prelaze 50% ukupne aktivnosti enzima, budući da čak i bolesnici s infarktom miokarda rjeđe imaju vrijednosti veće od 30%.
Točno otkrivanje makroenzima CK zahtijeva postupke koji omogućuju određivanje nihove molekularne mase (kromatografija, gradijentna gel elektroforeza), a opisano je i nekoliko testova probiranja na makroCK (37).

Zaključak

Svjesnost postojanja makroenzima, kao i postupaka njihovog određivanja ima, dakle, važnu ulogu u postavljanju dijagnoze, kako bi se izbjegla moguća primjena nepotrebnih, skupih i invazivnih dijagnostičkih postupaka. O dokazanoj prisutnosti nekog od makroenzima u serumu bolesnika potrebno je obavijestiti njegovog liječnika, kako bi se to dokumentiralo u njegovom zdravstvenom kartonu odnosno povijesti bolesti. S druge strane, treba uvjeriti i bolesnika da pojava takvog oblika enzima ne zahtijeva posebne terapijske mjere. Valja naglasiti da su podaci o mogućem dijagnostičkom značenju pojave makroenzima oskudni, nejasni i upitni. Ovu činjenicu ne treba zanemariti, nego informacije o razlozima prisutnosti makroenzima i mehanizmima njihova nastajanja treba nadalje pratiti i skupljati. Moguće je da će daljnja istraživanja u stvaranju i otkrivanju makroenzima u budućnosti rezultirati definiranjem nekih novih dijagnostičkih biljega.

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roCK type 2 association with malignant disease is direct enzyme release from malignant or necrotic cells (29). The occurrence of the macroenzyme need not always be associated with the increase in total CK activity, however, macroCK may interfere with some immunoinhibition procedures of CK-MB determination (2). Yet, it should be noted that macroenzymes CK should be considered in patients with CK-MB concentrations exceeding 50% of total enzyme activity, because values greater than 30% are rarely found even in patients with myocardial infarction.
The exact detection of macroenzyme CK requires procedures that enable determination of their molecular mass (chromatography, gradient gel electrophoresis). A number of screening tests for macroCK have been described (37).

Conclusion

Accordingly, awareness of the presence of macroenzymes and procedures for their determination should be an integral part of diagnostic work-up, in order to obviate the use of unnecessary expensive and invasive diagnostic procedures. The patient's physician should be informed on the presence of a macroenzyme in the patient's serum. These data should be entered in the patient's medical records and history form. On the other hand, the patient should be properly reassured that the occurrence of this enzyme form requires no specific therapeutic intervention.
It should be noted that data on the possible diagnostic significance of the macroenzyme occurrence remain scarce, vague and questionable. This fact should not be neglected, but the information on the reasons for the presence of macroenzymes and on the mechanisms of their formation should be continuously collected and reconsidered. Indeed, future research into the formation and detection of macroenzymes may hopefully result in defining some new diagnostic markers.

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