Molekularni mehanizmi i biokemijski biljezi akutnog pankreatitisa

Molecular mechanisms and biochemical markers of acute pancreatitis

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Sažetak

Unatoč nedavnim iskoracima u ispitivanju etiopatogeneze akutnog pankreatitisa (AP), još uvijek nisu objašnjeni svi čimbenici koji određuju nastup i tijek te bolesti. Istraživači se usredotočuju na ispitivanje čimbenika koji uvjetuju razvoj teškog AP, te razmatraju genetičku predispoziciju, vrstu mogućih odgovornih čimbenika kao i istodobno prisutna oboljenja. Najbitnija zadaća preostaje, međutim, procjena osjetljivosti i specifičnosti biokemijskih i seroloških biljega u dijagnozi i prognozi razvoja teškog AP te njihova klinička dostupnost. Aktivnost lipaze u serumu je pouzdaniji dijagnostički biljeg AP, nego aktivnost serumske amilaze. Tripsinogen-aktivacijski peptid (TAP) omogućava ranu dijagnozu AP. Korisni predskazatelji težine bolesti mogu uključivati prokalcitonin i TAP u mokraći, interleukine 6 i 8 u serumu, 24-satnu polimorfonuklearnu elastazu, te 48-satni C-reaktivni protein.

Istraživači se usredotočuju na potragu za najboljim mogućim prognostičkim čimbenikom AP. U kliničkoj se praksi, međutim, dijagnoza temelji na biološkim biljezima, kliničkoj procjeni i drugim laboratorijskim rezultatima koji su uključeni u Ransonovu ljestvicu ili APACHE II, te na slikovnim pretragama koje se koriste za određivanje pokazatelja težine bolesti kompjutorskom tomografijom (engl. *computer tomography severity index*, CTSI). Točnu prognozu tijeka AP nije moguće načiniti sve dok nisu određeni svi ti parametri kako bi se moglo započeti s najboljom mogućom terapijom.

Ključne riječi: akutni pankreatitis, lipaza, amilaza, prokalcitonin, CRP, interleukini

Abstract

Despite recent advances in research on etiopathogenesis of acute pancreatitis (AP), not all factors that determine the onset and course of the disease have been explained. Researchers concentrate on investigating the factors conditioning the development of severe AP. They consider genetic predisposition, the type of possible responsible factors, and comorbidities. The most essential task, however, remains to be the assessment of sensitivity and specificity of biochemical and serological markers in diagnosis and prognosis of developing severe AP and their clinical availability. Serum lipase is a more reliable diagnostic marker of AP than serum amylase. Trypsinogen activation peptide (TAP) ensures early diagnosis of AP. Useful predictors of severity may include serum procalcitonin and urinary TAP, serum interleukins-6 and -8 and polymorphonuclear elastase at 24 h, and serum C-reactive protein (CRP) at 48h.

Researchers concentrate on searching for the best possible prognostic factor of AP. In clinical practice, though diagnosis is based on biological markers, clinical assessment and other laboratory results included in Ranson scale or APACHE II and imaging investigations used to define computer tomography severity index (CTSI). Accurate prognosis of AP course cannot be made until all those parameters have been determined so that the best possible treatment can be undertaken.

Key words: acute pancreatitis, lipase, amilase, procalcitonin, CRP, interleukins

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Uvod

Usprkos nedavnim napredovanjima postignutima u istraživanju etiopatogeneze akutnog pankreatitisa (AP), još nisu razjašnjeni svi čimbenici koji određuju nastup i tijek te bolesti. Klasično se AP definira kao akutan upalni proces koji se razvija unutar gušteračne žlijezde s manjom ili većom uključenošću okolnog tkiva i/ili drugih organa (1).

Introduction

Despite recent advances in the research on etiopathogenesis of acute pancreatitis (AP), not all factors that determine the onset and course of the disease have been explained. Classically, AP is defined as an acute inflammatory process developing within the pancreatic gland with lesser or greater involvement of adjacent tissues and/

U većini je slučajeva to samoograničavajući proces, no u 20% oboljelih razvija se težak oblik nekroze s višeorganskim komplikacijama. Težak oblik AP uzrokom je do 50% smrtnih slučajeva (1). U slučaju pankreatične nekroze postoje dva razdoblja obilježena povećanom smrtnošću: prvo unutar prvih 7 dana od nastupa bolesti zbog višeorganskog zatajivanja, te drugo, kasnije razdoblje obično 2 tjedna nakon nastupa (kasna smrtnost) koje je najčešće uzrokovano infekcijama s lokalnim komplikacijama (npr. apscesi) ili općim infekcijama (npr. septikemija) (2).
Istraživači se usredotočuju na istraživanje čimbenika koji uvjetuju razvoj teškog AP, te uzimaju u obzir genetičku predispoziciju, vrstu mogućeg odgovornog čimbenika i prateća oboljenja. Eksperimentalna istraživanja pridaju veliku pozornost vrednovanju učinaka ishemije na razvoj AP (3,4). Ipak, najvažnija zadaća ostaje procjena osjetljivosti i specifičnosti biokemijskih biljega u prognoziranju rizika za razvoj teškog AP, te procjena njihove kliničke dostupnosti (5).
Najpoznatiji mehanizmi razvoja AP su, primjerice, aktivacija intrapankreatičnog enzima potaknuta aktivacijom tripsinogena u tripsin, nakon koje slijedi otpuštanje posrednika upale iz infiltrirane pankreatične vezivne strome - tj. citokina, adhezijskih molekula, čimbenika aktivacije trombocita (engl. *platelet activating factors*, PAF), dušičnog oksida (NO), reaktivnih oblika kisika, te lizosomskih enzima (6).

Molekularni mehanizmi AP

Uloga genetičkih čimbenika

Nedavno je istraživanje ukazalo da aktivaciji tripsinogena prethodi izmijenjena aktivacija miogenom aktivirane protein-kinaze (MAP), p38MAP-kinaze i c-jun aminoterminalne kinaze (JUN). Koncentracija p38MAP-kinaze raste najbrže, s vršnom koncentracijom nakon 3 sata. Aktivnost JUN-kinaze je najviša nakon 12 sati, a nakon 24 sata više se ne može detektirati (7,8).
Nuklearni transkripcijski faktor-κβ (NF-κβ) je poveznica između daljnjih stadija upalnog odgovora i imune reakcije te je primarni poznati regulator genskog izražaja mnogih posrednika upale. NF-κβ aktiviraju citokini, oksidacijski stres ili endotoksemija. NF-κβ se zatim premješta iz citoplazme u jezgru gdje se veže za specifične DNA-promotorske jedinice i potiče prijepis izvornih gena. Broj tako potaknutih posrednika upale dokazuje da je taj faktor uvelike uključen u pokretanje i širenje upalne reakcije s lokalne na opću razinu (9,10).

Uloga inhibitora proteolitičkih enzima

Gušterača u zdravom tijelu ima mnoge mehanizme koji je štite od samodigestije od svojih vlastitih enzima. Proteolitičke enzime, od kojih je tripsinogen najvažniji, stvaraju acinarne stanice kao proenzime, a u dvanaesniku ih

or other organs (1). In majority of cases it is a self-limiting process, yet 20% of patients develop severe form of necrosis with multiorgan complications. Severe form of AP accounts for up to 50% of mortality (1). In case of pancreatic necrosis, there are two periods of increased mortality: the one within the first 7 days from the onset due to multiorgan failure, and another, late period occurring commonly two weeks after the onset (late mortality) and usually due to infections with local complications (e.g. abscesses) or generalized infections (e.g. septicemia) (2).
Researchers concentrate on investigating the factors conditioning the development of severe AP. They consider genetic predisposition, the type of a possible responsible factor, and comorbidities. Experimental research pays much attention to the evaluation of ischemia effects on developing AP (3,4). The most essential task, however, remains to be the assessment of sensitivity and specificity of biochemical markers in prognosing the risk of severe AP development, and of their clinical availability (5).
The best known mechanisms of AP development are, e.g., intrapancreatic enzyme activation triggered by trypsinogen activation to trypsin followed by inflammatory mediators released from the infiltrated pancreatic connective stroma, i.e. cytokines, adhesive molecules, platelet activating factors (PAF), nitric oxide (NO), oxygen reactive forms (ORF) and lysosomal enzymes (6).

Molecular mechanisms of AP

The role of genetic factors

Recent research has suggested that trypsinogen activation is preceded by altered activation of myogen-activated protein kinase (MAP), p38 MAP kinase and c-jun amino-terminal (JUN) kinase. The level of p38MAP kinase increases most rapidly, with the peak of activity after three hours. JUN kinase activity is the highest after 12 hrs and after 24 hrs its activity becomes undetectable (7,8).
Nuclear transcription factor-κβ (NFκβ) is the link between subsequent stages of inflammatory response and immune reaction, and it is the primary known regulator of gene expression of many proinflammatory mediators. NF-κβ is activated by cytokines, oxidative stress or endotoxemia. NF-κβ is then translocated from the cytoplasm to the nucleus where NF-κβ binds specific DNA promoting items and induces transcription of proper genes. The number of thus triggered inflammatory mediators proves that it is highly involved in the initiation and expansion of inflammatory reaction from local to the generalized level (9, 10).

The role of proteolytic enzyme inhibitors

In a healthy body, the pancreas has many protective mechanisms against self-digestion by its own enzymes. Proteolytic enzymes, of which trypsinogen is the most im-

aktiviraju crijevne endopeptidaze. Enterokinaza iz površine membrane hidrolizira vezu Lys^{23}-Ile^{24} i otpušta krajnji oktapeptid, nazvan tripsinogen-aktivacijski peptid (TAP). I sam tripsin može aktivirati tripsinogen. Također, pankreatični sekrecijski tripsin-inhibitor (PSTI) štiti gušteraču od aktivacije tripsinogena u lobularnim stanicama. On se veže za tripsin u omjeru 1:1. Procjenjuje se da je molarni omjer PSTI i tripsina 1:10. Ako je aktivirano više od 10% tripsinogena, zaštitni mehanizmi više nisu učinkoviti (11). Osim PSTI, i sam tripsin može ograničiti svoju aktivnost razlaganjem veze Arg^{117} u molekuli tripsinogena. Usto su istraživanja potvrdila prisutnost izoforme ljudskoga tripsinogena, tj. mezotripsinogena koji je rezistentan na inhibitore tripsina. Ispitivanje njegove biološke funkcije je rezultiralo dvjema oprečnim teorijama: da mezotripsin može ili prerano aktivirati ili razgraditi pankreatične zimogene te time ili potaknuti ili zaštititi od patogeneze AP. Sposobnost mezotripsina da se veže za inhibitore uzrokovana je jednom mutacijom koja mezotripsinu pridaje jedinstvenu novu funkciju. Istraživači predlažu da biološka funkcija ljudskog mezotripsina uključuje razgradnju inhibitora tripsina u probavnom sustavu. Neadekvatna aktivacija mezotripsinogena u gušterači može pridonijeti razvoju AP (12).
Jedan od rijetkih uzroka AP je genetičko oboljenje koje je ovisno o genskoj mutaciji kationičkog tripsinogena. Genetičkim je istraživanjima otkrivena mutacija na poziciji 122 (R122H) gdje je histidin zamijenjen argininom (13). Ta je zamjena odgovorna za spontanu aktivaciju tripsina zbog autolize i počinje povezivanjem Arg^{122}-Lys^{123}. Jednom kad je tripsinogen aktiviran i njegova koncentracija nadmaši koncentraciju PSTI, aktiviraju se i ostali enzimi te se razvija AP. Za mutacije gena PSTI smatra se da preinačuju tijek AP time što smanjuju prag AP i povećavaju težinu upalnog procesa (14).

Biokemijski biljezi AP

Dijagnostički biljezi

Mnogi se biološki biljezi koriste za dijagnosticiranje i predviđanje težine AP. Ako se koriste usporedno s kliničkim simptomima, oni pomažu u dijagnozi i pokretanju pravodobnog liječenja. Najuobičajeniji biljezi uključuju lipazu i amilazu. Oba ta enzima luče pankreatične acinarne stanice (15,16). Njihov sadržaj u serumu ovisi o vremenu koje je proteklo od nastupa prvih pritužbi na bol i druge abdominalne simptome, koncentraciji triglicerida i drugim kroničnim stanjima kao što je npr. zatajivanje bubrega (17). Aktivnost amilaze može ostati normalna u bolesnika kod kojih postoji zlouporaba alkohola unatoč očiglednim kliničkim simptomima AP. Najveća se aktivnost tog enzima bilježi između 2 i 12 sati od nastupa simptoma i smanjuje se kako simptomi slabe.

portant one, are produced by acinar cells as proenzymes and activated in the duodenum by intestinal endopeptidases. Brush border enterokinase hydrolyzes Lys^{23}-Ile^{24} bond and releases the end-stage octapeptide called trypsinogen activation peptide (TAP). Trypsin itself can activate trypsinogen as well. Also, pancreatic secretory trypsin inhibitor (PSTI) protects the pancreas from trypsinogen activation in lobular cells. It binds to trypsin at the 1:1 ratio. The molar ratio of PSTI to trypsin is estimated to be 1:10. If more than 10% trypsinogen is activated, protective mechanisms are no longer effective (11). Apart from PSTI, trypsin itself may limit its activity by breaking down the Arg^{117} bond in a trypsinogen molecule. Additionally, investigations have confirmed the presence of human trypsinogen isoform, i.e. mesotrypsinogen resistant to trypsin inhibitors. The study of its biological function has produced two contradictory theories: that mesotrypsin may either prematurely activate or degrade pancreatic zymogenes, thus either triggering or protecting from the pathogenesis of AP. Mesotrypsin ability to bind inhibitors is due to a single mutation which gives mesotrypsin a unique new function. Researchers suggest that biological function of human mesotrypsin involves degradation of trypsin inhibitors in the alimentary tract. Improper mesotrypsinogen activation in the pancreas may contribute to the development of AP (12).
One of the rare causes of AP is genetic condition depending on cationic trypsinogen gene mutation. Genetic studies discovered mutation at 122 position (R122H), where histidine was replaced by arginine (13). The switch is responsible for spontaneous trypsin activation due to autolysis and it begins with Arg^{122}-Lys^{123} binding. Once trypsinogen gets activated and its level exceeds PSTI concentration, other enzymes also get activated and AP develops. Mutations of the PSTI gene are thought to modify the course of AP via decreasing the threshold of AP and increasing the severity of the inflammatory process (14).

Biochemical markers of AP

Diagnostic markers

Many biological markers are used to diagnose and predict the severity of AP. If convergent with clinical symptoms, they help in diagnosis and instituting prompt treatment. The most common markers include lipase and amylase. Both enzymes are secreted by pancreatic acinar cells (15,16). Their serum content depends on the time that has passed from the onset of first complaints of pain and other abdominal symptoms, triglyceride concentration and other chronic conditions, such as kidney failure (17). Amylase activity may remain normal in alcohol abusing patients despite evident clinical symptoms of AP. The peak of this enzyme activity occurs between the 2nd and

Mnogo osjetljivija i specifičnija jest aktivnost lipaze, osobito u bolesnika kod kojih postoji zlouporaba alkohola. Najveća aktivnost tog enzima prisutna je između 4 i 8 sati od nastupa simptoma. Danas je uobičajena praksa da se za dijagnosticiranje AP koristi jedino aktivnost lipaze, jer istodobno određivanje i aktivnosti amilaze ne osigurava veću dijagnostičku točnost (18). Sljedeći dijagnostički biljeg jest aktivnost alanin-aminotransferaze (ALT). Povišene aktivnosti koje koreliraju s aktivnošću amilaze i lipaze odgovorne su za 95%-tnu žučnu etiologiju AP. Vrijednosti > 150 mg/L smatraju se graničnima. Tripsinogenom aktivirani protein (TAP) u mokraći je koristan biljeg u dijagnozi i procjeni težine AP. Povećane vrijednosti tog biljega zapažaju se nekoliko sati (6-12) nakon nastupa simptoma no, na žalost, on jedva da je klinički koristan zbog svoje ograničene raspoloživosti (19).

Iako su nalazi u povijesti bolesti najvažniji u slučaju AP povezanog s alkoholom, dijagnozu u dvojbenim slučajevima olakšava određivanje disijalotransferina u krvnom serumu koji predstavlja tipičan biljeg izražene zlouporabe alkohola. Ako se izmjeri 24 sata nakon prijama, taj biljeg povećava vjerojatnost dijagnoze AP povezanog s alkoholom od 64% na 94% (20).

Prognostički biljezi

Uloga C-reaktivnog proteina

Predviđanje težine bolesti je važno u obradi bolesnika s AP. S time u vezi najuobičajeniji biološki biljeg jest C-reaktivni protein (CRP). CRP je protein akutne faze kojeg obilato stvaraju hepatociti. To stvaranje potiču citokini kao što su interleukin 6 (IL-6), čimbenik nekroze tumora alfa (engl. *tumor necrosis factor alpha*, TNF-α) i interleukin 1-beta (IL-1beta) (21). Pojačana aktivnost CRP je usko povezana s težinom AP i tendencijom razvoja nekroze gušterače. Granična vrijednost iznosi 150 mg/L. Na žalost, koncentracija CRP značajno raste 24-48 sati nakon nastupa bolesti (19,22) te je stoga beskoristan tijekom prvih 24 sata (23). Osjetljivost CRP je 37-77% kod dokazivanja teškog AP unutar 24 sata i raste sve do 83-100% 48 sati nakon pojave prvih simptoma (24). Slične rezultate je dobio i Gürleyik. On je utvrdio da je osjetljivost CRP 48 sati nakon nastupa bolesti bila 84%, specifičnost 73%, a pozitivna prediktivna vrijednost (PPV) 50,1% (25).

Kako se za osjetljivost CRP ispostavilo da je vrlo mala unutar 24 sata od nastupa bolesti, potraga za ranim biljezima usmjerena je prema citokinima. Radi se o skupini različitih peptida koji pokazuju signalne karakteristike. Skupina se dijeli u monokine, limfokine, kemokine, hematopoetine i interferone. Ti proteini često pokazuju biološka svojstva iako imaju i neke zajedničke osobine: aktivni su u vrlo malim koncentracijama, ne stvaraju se neprekidno, no stanice se potiču na njihovu sitnezu, imaju biološke učinke

12th hr from the onset of symptoms and decreases as the symptoms subside.

Much more sensitive and specific is the activity of lipase, particularly in the alcohol abusing patients. The peak of its activity occurs between the 4th and 8th hr from the onset of symptoms. A common practice nowadays is to use lipase activity alone to diagnose AP since concomitant determination of amylase activity does not provide higher diagnostic accuracy (18). Another diagnostic marker is alanine aminotransferase (ALT) activity. Increased values correlating with amylase and lipase activity account for 95% billiary etiology of AP. The values higher than 150 mg/L are assumed to be cut-off values. Urine trypsinogen activated protein (TAP) is a useful marker to diagnose and assess the severity of AP. Increased values are observed several hours (6-12) after onset of symptoms. Unfortunately it is hardly clinically useful due to its limited availability (19).

Although medical history findings are most important in case of alcohol AP, in doubtful cases the diagnosis is easier when blood serum disialotransferrin is determined, a specific marker of high alcohol abuse. If determined after 24 hrs from admission, it increases the probability of diagnosing alcohol AP from 64% to 94% (20).

Prognostic markers

The role of C-reactive protein

Prognosing disease severity is important in managing patients with AP. The most common biological marker in that regard is C-reactive protein (CRP). CRP is an acute-phase protein abundantly produced by hepatocytes. The production is stimulated by cytokines, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and interleukin 1beta (IL1-beta) (21). Enhanced CRP activity correlates closely with the severity of AP and the tendency of developing necrosis of the pancreas. The cut-off value is 150 mg/L. Unfortunately, CRP concentration increases significantly 24-48 hrs after the onset of disease (19, 22). Therefore it is useless within the first 24 hrs (23). CRP is 37-77% sensitive in confirming severe AP within 24 hrs and its sensitivity increases up to 83-100% 48 hrs after the occurrence of first symptoms (24). Gürleyik has obtained similar results. He found that the CRP determined 48 hrs after disease onset was 84% sensitive, 73% specific, and had 50.1% positive predictive value (PPV) (25).

Since CRP turned out to have little sensitivity within 24 hrs from the disease onset, the search for early markers turned to cytokines. It is a group of various peptides that exhibit signaling characteristics. The group is divided into monokines, lymphokines, chemokines, hematopoetins and interferons. These proteins often exhibit different biological properties though they have some common

vezanjem za visokospecifične receptore na ciljanoj staničnoj površini, a imaju i plejotrofno djelovanje (2,6).

Uloga citokina

Za prognozu AP najvažniji je IL-6 kojega stvaraju monociti, makrofagi, limfociti T i B, endotel, epitel i fibroblasti, hondroblasti i osteociti. Koncentracija mu raste između 18 i 48 sati od nastupa bolesti. IL-6 predstavlja čimbenik koji započinje stvaranje proteina akutne faze u jetri (CRP) i pojačava sintezu IL-2 i njegova receptora na površini T-limfocita. Suprotno od ostalih citokina, lako ga se otkriva u cirkulaciji i pokazuje tipičnu endokrinu aktivnost (2,26). Jiang je utvrdio da je nakon 24 sata IL-6 pokazao veću osjetljivost (100%), specifičnost (89,9%) i PPV (91%) nego CRP i TNF-α, čime je naglašena njegova korisnost u procjeni težine bolesti tijekom prvih 24 sata (27).

Drugi važan citokin je IL-8. Taj kemokin stvaraju brojne stanice opremljene s TNF-α i IL-1-receptorima. Vjerojatno je da su acinarne stanice gušterače primaran izvor IL-8 i ostalih citokina (28). Berney je utvrdio da je koncentracija IL-8 u serumu povezana s težinom bolesti, posebice kad upala organa postane opći proces (29). Najviše su mu koncentracije zapažene između 12 i 24 sata od nastupa bolesti (5). Slično kao i ostali kemokini, IL-8 pretvara djelomično neaktivne integrine u molekule s aktivnom konfiguracijom (26). IL-8 stoga može biti vrlo koristan u procjeni težine AP u ranom stadiju tog oboljenja.

IL-15 je citokin koji ima mnoga biološka svojstva IL-2. Njegove su koncentracije ispitivane u odnosu na težinu AP, višeorganske komplikacije i infekcije. Koncentracija tog citokina bila je visoka u bolesnika s disfunkcijom više organa, infekcijama te u bolesnika koji su umrli tijekom AP. IL-15 je korisniji od CRP, IL-6 ili IL-8 u predviđanju težine AP (30).

Član skupine monokina IL-18 također se, u odnosu na svoje blaže oblike, oslobađa u značajno višim koncentracijama u ranim stadijima pankreatične nekroze tijekom AP (31). IL-18 može aktivirati pomoćničke limfocite Th_1 i B-limfocite te jača obranu od infekcija. S druge strane on potiče otpuštanje TNF-α, kemokina i INFγ. Ukazano je da IL-18 djeluje kao značajna poveznica u poremećenom imunom odgovoru kod nekrotičkog oblika AP.

Kao i IL-18 iz skupine monokina, TNF-α je biološki biljeg koji se razmatra kod procjene težine AP. Tijekom AP oslobađa se u jetri, plućima i slezeni. TNFα i IL-1 su glavni i najvažniji posrednici upale u AP (26). TNFα je ključni regulator proupalnih citokina i molekula adhezije leukocita. Može biti koristan u procjeni težine bolesti i indvidualne sklonosti za razvijanje organskih komplikacija i septičnog šoka. TNFα se, međutim, brzo ispire iz seruma tako da njegova osjetljivost i PPV uvelike ovise o vremenskom podudaranju s prvim simptomima (32). S obzirom da se čini da je TNFα ključni citokin u razvoju AP, on je također postao važnim ciljem terapije. Prvi članci istraživača etanercepta, inhibitora TNFα koji se primjenjuje u liječenju AP u mišefeatures: they are active in very small concentrations, they are not produced all the time but cells are stimulated to synthesize them, they produce biological effects via binding with highly specific receptors on the target cell surface, and they have pleiotrophic effects (2,6).

The role of cytokines

Most important for the prognosis of AP is IL-6, produced by monocytes, macrophages, lymphocytes T and B, endothelium, epithelium and fibroblasts, chondroblasts and osteocytes. Its concentration increases between 18 and 48 hrs from disease onset. It is a factor initiating the production of acute-phase proteins in the liver (CRP) and enhancing the synthesis of IL-2 and its receptor on T- lymphocyte surface. Contrary to other cytokines, it is detectable easily in the circulation, and it exhibits typical endocrine activity (2, 26). Jiang found that after 24 hrs IL-6 demonstrated higher sensitivity (100%), specificity (89.9%), and PPV (91%) than CRP and TNF-α, which emphasizes its usefulness in assessing disease severity within first 24 hrs (27).

IL-8 is another important cytokine. This chemokine is produced by numerous cells equipped with TNF- α and IL-1 receptors. Acinar cells of the pancreas are likely to be the primary source of IL-8 and other chemokines (28). Berney found that IL-8 concentration in serum correlated with disease severity, especially when organ inflammation became a generalized process (29). Its highest concentrations were observed between 12 and 24 hrs from disease onset (5). Similarly to other chemokines IL-8 converts partially inactive integrines into molecules of active configuration (26). Thus, IL-8 may be very useful to assess the severity of AP in its early stage.

IL-15 is a cytokine that has many biological properties of IL-2. Its concentrations have been examined with reference to AP severity, multiorgan complications and infections. Its level was high in patients with multiorgan dysfunction, infections and those who died during the course of AP. IL-15 is more useful than CRP, IL-6 or IL-8 to predict AP severity (30).

IL-18, a member of the monokine family, is also released at significantly higher concentrations in early stages of pancreatic necrosis in the course of AP as compared to mild forms (31). IL-18 can activate helper lymphocytes Th_1 and B-lymphocytes and enhances defense against infections. On the other hand, it stimulates the release of TNF-α, chemokine, and INFγ. It has been suggested that IL-18 acts as a significant link in disordered immune response in a necrotic form of AP.

TNFα, like IL-18 from the monokine family, is a biological marker considered in the assessment of AP severity. In the course of AP it is released in the liver, lungs and spleen. TNFα and IL-1 are the main and most important inflammatory mediators in AP (26). TNFα is the key regu-

va, već su objavljeni no, unatoč obećavajućim rezultatima, nužna su daljnja promatranja (33).

Ostali prognostički čimbenici

Prokalcitonin (PCT) je također koristan biljeg u procjenjivanju težine AP. Povećane koncentracije PCT u serumu bilježe se između 24 i 36 sati, osobito u bolesnika s inficiranom nekrozom (34). Takvo je zapažanje potvrđeno istraživanjem u više centara u kojima su uspoređene koncentracije PCT s koncentracijama CRP. Oba su parametra praćena uzastopno 21 dan. Za razliku od CRP, koncentracija PCT je bila značajno povišena u slučaju pankreatične nekroze, u bolesnika sa sindromom višeorganske disfunkcije (engl. *multiorgan dysfunction syndrome*, MODS), u svih bolesnika kod kojih je bio nužan naknadan kirurški zahvat, te u onih koji su umrli od AP. Istraživači su također ukazali na određivanje PCT kao jedinstvenog parametra rizika za razvoj komplikacija (35,36).

Fosfolipaza A2 je biljeg koji je povezan ne samo s pankreatičnom nekrozom već i s pulmonarnim komplikacijama tijekom AP (37). Visoke vrijednosti u serumu određuju se 24 sata nakon nastupa AP.

Prisutnost plućnih komplikacija je također povezana s matriks-metaloproteinazom-9 (MMP-9) koja se rano pojavljuje u krvnom serumu. Karakterizira ju negativna prediktivna vrijednost (NPV) od 96,2% i PPV koji dostiže 100% (38,39).

U istom je kontekstu ispitivan inhibicijski čimbenik migracije makrofaga (engl. *macrophage migration inhibitory factor*, MIF). On može pojačati upalnu reakciju uključenjem citokina na mjestu upale. Njegove su visoke vrijednosti zapažene 24 sata nakon nastupa AP u bolesnika u kojih se razvila pankreatična nekroza; taj čimbenik, međutim, nije korelirao s višeorganskim komplikacijama (40).

Povećane vrijednosti topljivog trombomodulina (sTM) pojavljuju se iza visokog MIF. Zapažene su nakon 48 sati u serumu bolesnika kod kojih postoji rizik razvoja pankreatične nekroze (41).

Čimbenik aktivacije trombocita (engl. *platelet-activating factor*, PAF) je proupalni fosfolipid iz biološki aktivne skupine triglicerida. Ti spojevi sudjeluju u procesima cijeljenja rana, angiogenezi i apoptozi, uključujući razvoj upale u tijeku AP (42).

Još jedan rani prognostički biljeg AP je polimorfonuklearna elastaza. Njena osjetljivost u prvih 24 sata hospitalizacije bolesnika kod predviđanja teškog oblika AP iznosi 92%, a specifičnost 91%. Polimorfonuklearna elastaza pokazuje 78%-tni PPV i 96%-tni NPV. Sve te karakteristike čine taj parametar vrijednim prognostičkim biljegom AP. Usto, on je lako dostupan u kliničkoj praksi (43).

Istraživači se usredotočuju na potragu za najboljim mogućim prognostičkim čimbenikom AP. U kliničkoj se praksi, međutim, dijagnoza temelji na biološkim biljezima, klinič-

lator of proinflammatory cytokines and leukocyte adhesion molecules. It might be useful in estimating the severity of disease and individual predisposition to develop organic complications and septic shock. TNFα, however, is washed out from serum rapidly so that its sensitivity and PPV depend closely on timing with the first symptoms (32). Since TNFα seems to be the key cytokine in developing AP, it has also become an important target of therapy. First research articles on etanercept, a TNF-α inhibitor applied in treatment of AP in mice, have been published but, despite promising results, further observation is necessary (33).

Other predicting factors

Procalcitonine (PCT) is also a useful marker to assess AP severity. Increased serum PCT concentrations occur between 24 and 36 hr, particularly in patients with infected necrosis (34). This observation has been confirmed in a multiple-center investigation which compared PCT levels with CRP. Both parameters were monitored for 21 consecutive days. Contrary to CRP, the level of PCT was significantly increased in case of pancreatic necrosis in patients with multiorgan dysfunction syndrome (MODS), in all patients who required subsequent surgery and in those who died of AP. Researchers also suggested the determination of PCT as a single parameter of the risk of developing complications (35,36).

Phospholipase A2 is a marker related not only to pancreatic necrosis but also to pulmonary complications in the course of AP (37). High blood serum values are determined 24 hrs after the onset of AP.

The presence of pulmonary complications is also related to matrix metalloproteinase-9 (MMP-9) occurring early in the blood serum. It is characterized by negative predictive value (NPV) of 96.2% and PPV reaching 100% (38,39).

Macrophage migration inhibitory factor (MIF) has also been investigated in this respect. It can intensify inflammatory reaction by cytokine involvement at the place of inflammation. Its high values were noted 24 hours after the onset of AP in patients who developed pancreatic necrosis; however, it did not correlate with multiple organ complications (40).

Increased values of soluble thrombomodulin (sTM) appear second to high MIF. After 48 hrs they were observed in the blood serum of patients at a risk to develop pancreatic necrosis (41).

Platelet-activating factor (PAF) is a proinflammatory phospholipid from the biologically active triglyceride family. These compounds take part in wound healing processes, angiogenesis and apoptosis, including the development of inflammation in the course of AP (42).

Another early prognostic marker of AP is polymorphonuclear elastase. Its sensitivity and specificity in the first 24

koj procjeni i ostalim laboratorijskim rezultatima uključenima u Ranson-ljestvicu ili APACHE II, te na slikovnim ispitivanjima koja se koriste da bi se definirao kompjutorsko-tomografski pokazatelj težine bolesti (CTSI). Točnu prognozu tijeka AP nije moguće postaviti sve dok nisu određeni svi ti parametri tako da se može započeti s najboljom mogućom terapijom.

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hrs of patients' hospitalization in predicting severe form of AP are 92% and 91%, respectively. Polymorphonuclear elastase demonstrates 78% PPV and 96% NPV. All these characteristics make this parameter a valuable prognostic marker of AP. Besides, it is easily accessible in clinical practice (43).

Researchers concentrate on searching for the best possible prognostic factor of AP. In clinical practice, though, diagnosis is based on biological markers, clinical assessment and other lab results included in Ranson scale or APACHE II and imaging investigations used to define computer tomography severity index (CTSI). Accurate prognosis of AP course cannot be made until all those parameters have been determined so that the best possible treatment can be undertaken.

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