

The role of pentraxin 3 in the inflammatory and immune response

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SUMMARY

Pentraxin 3 is the first identified and to date the most important protein of a recently recognized group called long pentraxins. In many aspects, the structure and function of PTX3 resembles that of short (or classical) pentraxins, i. e., C-reactive protein and the serum amyloid P component. There are, however, several important differences between the two groups of pentraxins that will be mentioned in more detail in the article. All of the above pentraxins take an active part in first-line defense of the host against invading pathogenic organisms and in the clearance of the host's own apoptotic cells. The latter mechanism impedes the onset of destructive autoimmune reactions. A biologically relevant antipode of PTX3 is TNF- α . The physiological course of the defense reactions depends on the closely coordinated activity of both peptides. In case of an unchecked or missing activity of either peptide, a disturbance in their mutual balance results in increased susceptibility of the host to conditionally pathogenic fungi or in increased damage to the host's own tissues inflicted by the defense reactions. This review article deals with the physiopathological importance of PTX3 on the basis of most recent information.

Key words: pentraxin 3, C-reactive protein, cytokines, complement system, apoptosis, dendritic cells, T-lymphocytes, *Aspergillus fumigatus*.

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PENTRAXINS: ACUTE PHASE PROTEINS

Pentraxin 3 (PTX3), occasionally also referred to as TSG-14 (**TNF-stimulated gene 14**), is a member of a constantly growing family of proteins collectively called pentraxins. From the evolutionary viewpoint, pentraxins are very old protein structures, whose form has basically remained unaltered ranging from simple invertebrates such as the crustacean *Limulus polyphaemus* to the most complex vertebrates including man. In terms of structure, all pentraxins exhibit a cyclic multimeric form. In terms of function, pentraxins are involved in the reactions of innate, antigen non-specific immune response and in the clearance of cells dying from apoptotic death. Increased interest in pentraxins which is experienced in recent years results from their incontestable involvement in the pathogenesis of atherosclerosis (1).

“Classical” or “short” pentraxins, known for several decades, include C-reactive protein (CRP) and serum amyloid P component (SAP). CRP is a representative of acute phase proteins that are produced in the liver under the control of proinflammatory cytokines, in particular of IL-6. In an acute phase reaction, plasma CRP levels quickly rise to hundred to thousand folds of baseline concentrations. SAP is an acute phase reactant in small rodents such as the mouse while, in man, plasma levels of SAP are relatively constant and their increase in the systemic inflammatory response is quantitatively of little significance. CRP and SAP bind structures (ligands) to their target cells or other target particles mostly—though not exclusively—in

the presence of calcium ions. Prototypical ligands of classical or short pentraxins include choline phosphate, ethanolamine phosphate, DNA and nuclear chromatin of dying cells, immune complexes, several components of the complement system, and proteins of the extracellular matrix. As the body of knowledge about atherosclerosis as a systemic condition with an inflammatory and an (auto)immune component increases, so does the body of knowledge about the role of CRP as a biomarker and a mediator of cardiovascular diseases, that is, not only of coronary heart disease but, also, arterial hypertension, congestive heart failure and ischemic stroke (2).

PTX3 STRUCTURE: SIMILARITIES WITH AND DIFFERENCES FROM CLASSICAL PENTRAXINS

The TSG-14 gene product has been obtained as the quantitatively most important protein produced by human fibroblasts stimulated *in vitro* by TNF- α . Independent of this finding yet almost at the same time, a practically identical protein (with the only exception, i. e., the presence of methionine instead of leucine at position 202 of the amino acid chain) has been identified after stimulation of human endothelial cells with IL-1 β . The resulting product was called pentraxin 3 (PTX3). The **C-terminal** end of the PTX3/TSG-14 protein displays remarkable similarity (*homology*) with classical pentraxins, C-reactive protein and serum amyloid P component. The **N-terminal**

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end of PTX3/TSG-14 does not resemble any protein known so far. The cDNA corresponding to PTX3/TSG-14 encodes a protein consisting of 381 amino acid residues, 17 of which constitute a signal sequence that is split off during the process of secretion of the final product. The C-terminal "pentraxin" domain includes 203 amino acid residues connected with a unique N-terminal end, consisting of 161 amino acid residues, which is completely different from the classical pentraxins. After the signal peptide sequence has been split off, the secreted PTX3/TSG-14 protein gains a molecular weight of 42 kDa. The molecular weight of the final protein after complete glycosylation is 47 kDa. Consistent with their classification as pentraxins, separate PTX3 units form structures of higher order, most frequently decamers (ten PTX3 units) or dodecamers (twenty PTX3 units) (3).

PTX3 PRODUCTION BY ORGANS AND CELLS

In human serum or plasma under baseline conditions, PTX3 levels are close to the limit of detection by current laboratory methods. Unlike classical pentraxins, PTX3 formation in hepatocytes is negligible. Similarly, IL-6 exerts virtually no control over PTX3 formation. By contrast, PTX3 is produced in other organs, especially in the heart and striated muscle, as well as in the lungs, ovarium, thymus, and the skin. In cell cultures, PTX3 is produced by TNF- α or IL-1 β -stimulated fibroblasts, endothelial cells, monocyte-macrophage cell lines and by the dendritic cells. Additionally, in monocyte-macrophage cell lines, PTX3 formation is enhanced by bacterial lipopolysaccharide and by components of mycobacterial cell membranes referred to as liparabinomans. PTX3 formation in cells of the monocyte-macrophage lineage is inhibited by IFN- γ (4).

PTX3 AND THE COMPLEMENT SYSTEM

Unlike classical pentraxins, PTX3 features a somewhat different spectrum of binding ligands: it does not bind most of the structures that are typically bound by classical pentraxins with the use of calcium ions. Hence, PTX3 does not bind choline phosphate, the main binding structure of C-reactive protein. On the other hand, PTX3 binds with high affinity the C1q factor of complement, or the C1qC1rC1s complex. Binding between PTX3 and C1q exhibits the nature of competitive inhibition: in a milieu with excess competitors, i. e. CRP or SAP, binding of C1q by PTX3 is inhibited. If such is the case, C1q is bound by the short pentraxins instead of by PTX3. The milieu in which binding between PTX3 and C1q occurs determines the final outcome of the interaction, which may be either activation or inhibition of the classical pathway of the complement cascade. PTX3 generated directly in tissue activates the complement system. This form of activation occurs locally, with complement activity remaining limited to the focus of inflammatory reaction. On the other hand, if levels of circulating PTX3 increase systemically, the classical pathway of complement activation is inhibited by PTX3. The mechanism of this inhibition consists of an insertion of the PTX3 molecule between C1q and the following factors of the complement cascade. In the bloodstream, factor C1q is thus virtually isolated by PTX3 from the subsequent complement components. This is a mechanism by which PTX3 prevents undesirable systemic complement activation in the course of an inflammatory response (5).

MACROPHAGE SECRETORY PHENOTYPE

Normal cellular homeostasis requires constant clearance of old and damaged cells dying from apoptotic death. Apoptotic cells are a "reservoir" of potentially dangerous autoantigens, particularly of

intracellular organelles and nuclear chromatin. In tissues with an ongoing inflammatory response, the rate of apoptotic cell death rises many times. While pathogenic microorganisms are phagocytosed by macrophages that had been activated by the proinflammatory cytokines TNF- α , IL-1 β and/or IFN- γ , phagocytosis of the host's own apoptotic cells results in a shift of the macrophages' proteosynthetic and secretory phenotype to the formation and secretion of anti-inflammatory cytokines IL-10 and TGF- β . The host's own viable cells are thus protected against damage inflicted by the defense inflammatory reactions (bystander damage). The outcome of the inflammatory reaction—elimination of pathogenic microorganisms versus damage to the host's own tissues—depends on the interaction of both seemingly opposing processes (6, 7).

APOPTOSIS AND DENDRITIC CELLS

Old and damaged cells of the host's own tissues are phagocytosed not only by macrophages but, also, by dendritic cells. Together with monocytes/macrophages, B-lymphocytes and endothelial cells, dendritic cells belong to so-called antigen-presenting cells (APCs). Dendritic cells constitute the link between the innate (antigen-nonspecific) and adaptive (antigen-specific) immune response, as they are the only cell population capable of presenting antigens to so-called "naïve" or non-committed T-lymphocytes. These Th0 lymphocytes are essential for the cellular or humoral immune response to antigens that the body is naïve to, hence, no memory T-lymphocytes or B-lymphocytes are available (8).

Dendritic cell precursors originating in bone marrow carry, in their membrane, the differentiating antigen CD34. Blood flow transports these cells to individual organs where they colonize the tissue interstitium (9). Once deposited there, CD34+ precursors turn into immature dendritic cells that perform local immunological surveillance: they take up, with great efficacy, foreign antigens including pathogenic microorganisms (10). For antigen uptake, dendritic cells use receptors located in their membranes. The most important membrane receptors include:

- Receptors for the Fc domains of immunoglobulins called Fc γ RI, Fc γ RII, and Fc γ RIII, and complement fragment receptors, particularly the CR3 receptor, serving to phagocytose immune complexes and bacteria,
- So called "toll-like" receptors which are involved in the identification of biologically active structures present either in bacterial membranes and or in a soluble form that are shared by various pathogenic microorganisms (lipopolysaccharides, peptidoglycan, lipoteichoic acid, CpG fragments of bacterial DNA),
- The scavenger receptor CD36 and the integrins α v β 3 and α v β 5, used for phagocytosis of apoptotic cells (11-13).

ANTIGEN PRESENTATION BY DENDRITIC CELLS

After antigen uptake, dendritic cells migrate to lymphatic vessels cells by which they get to regional lymphatic nodes. Maturation of dendritic cells occurs during their migration. A role in the process of maturation is played, in addition to contact of dendritic cells with the antigen, by the proinflammatory cytokines TNF- α , IL-1 β and IFN- γ (14). By contrast, maturation of dendritic cells is inhibited by anti-inflammatory agonists such as IL-10, TGF- β , some prostaglandins, and corticosteroids (15, 16). While losing their ability to take up antigens, mature dendritic cells gain the ability to present, on their surface, antigenic peptides bound to MHC class I or II molecules (17). The process of maturation is accompanied by expression of costimulation molecules CD80 or B7-1 and CD86 or B7-2 (18). The complex

of antigen peptide + MHC class I or II that is presented by dendritic cells to the specific T-lymphocyte receptor (TCR), generates an antigen-specific "signal 1", which activates the host's own immune response. The interaction of costimulating molecules, presented by dendritic cells to their corresponding counterparts (CD28) on the surface of T-lymphocytes (CD80-CD28, CD86-CD28), generates an antigen-non-specific "signal 2" (19). The second signal prolongs the time necessary for complete activation of the immune response, e. g., by delaying apoptosis of APCs (20). Isolated presentation of antigen without concomitant costimulation results in premature death of dendritic cells and antigen-specific T-lymphocytes. This may be one of the mechanisms whereby dendritic cells prevent the development of autoimmune reactions against antigens originating from their own dying cells. Autoantigen presentation without costimulation triggers local deletion of autoreactive T-lymphocytes (21). An important role in the development of the immune response is played by the CD40/CD40L complex, perhaps the most important intercellular communication system operating not only in the activation of immune reactions but, also, in the pathogenesis of atherosclerosis. Binding of the protein CD40 which is present on the surface of APCs with the corresponding counterpart on the surface of T-lymphocytes, i. e. the CD40L (L: ligand, the terminologically proper name instead of CD40L is CD154), will enhance the activation of both cell types, i. e., the dendritic cells and T-lymphocytes, including the formation and secretion of proinflammatory and anti-inflammatory cytokines (22). These complicated two-way signals, giving the final shape to the immune response, are provided for through a signal pathway that leads to activation of the nuclear transcription factor, NF- κ B (23).

APOPTOTIC CELL DEATH IN THE INFLAMMATORY REACTION

Under normal circumstances, cells of the host's own tissues die in the absence of proinflammatory signals, which would otherwise lead to dendritic cell maturation. However, during an inflammatory response, the host's own cells die in a milieu with increased levels of proinflammatory cytokines (24). The inevitable consequence is a manifold increase in the rate of dendritic cell maturation. Antigens presented by mature dendritic cells activate T-lymphocytes, triggering the immune response against their carriers, pathogenic microorganisms in most cases. Still, even under these circumstances, the immune response is specifically targeted at foreign antigens, instead of to being targeted to autoantigens derived from the host's own cells, whose influx is increased many times due to accelerated apoptosis. Thus, in a milieu flooded by proinflammatory cytokines, there must be protective agents operating to prevent phagocytosis of the host's own dying cells by mature dendritic cells with subsequent development of autoimmune reactions (25). In addition to the above mentioned proinflammatory cytokines, these protective agents include pentraxin 3.

PTX3 AND PROTECTION OF APOPTOTIC CELLS

Local production of PTX3 begins before the synthesis of classical pentraxins by hepatic parenchyma cells. PTX3 production is carefully controlled also in terms of the time course: it remains confined to the phase of apoptosis associated with autoantigen clustering in cell membranes, in so called apoptotic blebs. PTX3 thus does not bind to cell membranes until the late phase of apoptosis, later than annexin V or β 2-GPI (β 2-glycoprotein I). Apoptotic cells with PTX3 present in their membranes are protected against phagocytosis by mature dendritic cells. PTX3 per se has no effect on the pro-

cess of dendritic cell maturation (26). Autoantigen presentation to T-lymphocytes by immature dendritic cells in the presence of PTX3 maintains the immune tolerance of cells of the host's own tissues even in a milieu with an enhanced inflammatory reaction. PTX3 binding to apoptotic cell membranes displays features of competitive inhibition: PTX3 may be displaced from the bond by excess CRP or SAP. At the systemic level, both classical pentraxins play a role identical to that played by PTX3 at the local level: they support phagocytosis of the host's own apoptotic cells without concomitant activation or, alternatively, in the presence of active inhibition of inflammatory reactions (27). All three pentraxins likewise provide for the binding of the complement factor C1q to apoptotic blebs. The C1q factor is the only component of the complement system generated not only by hepatocytes but, also, by macrophages and immature dendritic cells. In terms of quantity, extrahepatic production of factor C1q is more relevant compared with its synthesis by hepatic parenchymal cells (28). Factor C1q triggers complement activation in a classical manner. Complement fragments C3b/iC3b, acting as opsonins that inhibit the inflammatory response, accumulate in conjunction with pentraxins on the surface of a material designed to be phagocytosed (29). The role of opsonins is also played by all three pentraxins, i. e., not only CRP and SAP but, also, by PTX3 (30). If pentraxins are absent in the given milieu, the complement becomes fully activated. Terminal complement complexes C5b-9(n), enhancing the inflammatory response, accumulate on the surface of the material being phagocytosed.

PTX3 REGULATORY EFFECTS ON THE INFLAMMATORY RESPONSE

While CRP and SAP are produced in the liver and carried to the foci of inflammatory reaction by blood flow, PTX3 is formed locally, at site of ongoing inflammatory reaction. At the organ level, the most important contributors to PTX3 formation include the myocardium and skeletal muscle, even in cases whereby the primary inflammatory focus is located in a different organ, e. g., in the brain (31). PTX3 generated under the control of TNF- α , IL-1 β and/or the products of microbial degradation acts as a sensitive regulator of local reactions of the inflammatory and immune responses. The initial knowledge about the effect of PTX3 on the course of defense reactions was obtained from transgenic mice expressing increased levels of PTX3 (PTX3 Tg: transgene), or in so-called "knock-out" mice with PTX3 gene deletion ("PTX3 -/-", "PTX3-null").

PTX3 IN THE INFLAMMATORY RESPONSE: PROTECTIVE ACTION

Transgenic mice, producing increased levels of PTX3 following inflammatory stimulation, are resistant to the effects of bacterial lipopolysaccharide and to sepsis induced by caecal ligation and puncture. This model (caecal ligation and puncture, CLP) results in invasion of mixed bacterial flora to the peritoneal cavity imitating, with a high degree of accuracy, polymicrobial bacteremia in man, associated with relatively low plasma lipopolysaccharide levels but, also, high proinflammatory cytokine levels. Constitutive (baseline, nonstimulated) PTX3 generation in transgenic mice is comparable with that of control animals. Still, transgenic mice show increased baseline production of IL-10, the main anti-inflammatory cytokine. Inflammatory stimulation in transgenic mice, induced by intraperitoneal lipopolysaccharide administration or CLP, is followed by an massive increase in PTX3 production, primarily in myocardial and striated muscle endothelial cells, but not in hepatic parenchyma cells. Compared with control animals, the increase in plasma PTX3

levels is associated with a more rapid decrease in IL-10 levels and a more rapid increase in TNF- α levels. Adequate TNF- α activity, which is not counter-balanced by opposing effects of IL-10, is believed to play, in the initial phases of peritonitis, a pivotal role in mobilizing the first-line defense, represented by granulocytes, tissue macrophages, thrombocytes and fibroblasts. Appropriate in time and intensity, the activity of TNF- α results in inflammatory stimulation of the above cells, which become capable of phagocytosing pathogenic microorganisms. Peritoneal macrophages of transgenic mice, pre-stimulated by TNF- α , IFN- γ and/or bacterial lipopolysaccharide, generate high amounts of nitric oxide. Induced NO production enhances the body's bactericidal potential and, if confined to inflammatory foci, it additionally improves local hemodynamic conditions including faster remission of the inflammatory reaction. In the late phases of peritonitis, IFN- γ stops further PTX3 production by cells of the monocyte-macrophage lineage. Macrophage activation is decreased back to baseline level. The inflammatory reaction is confined both in terms of duration and location (32).

PTX3 IN THE INFLAMMATORY RESPONSE: DESTRUCTIVE ACTION

The situation is completely different with a sterile inflammation exemplified by reperfusion of an ischemic organ whereby PTX3—similar to C-reactive protein—increases not only organ damage but, also, overall mortality in involved individuals. PTX3-transgenic mice will again serve as an illustrative example. Occlusion of the arteria mesenterica superior for 60 minutes in the above animals will induce intestinal ischemia and 30-minute reperfusion following arterial declamping leads to the development of typical ischemia-reperfusion injury. In transgenic and control animals, reperfusion creates damage not only to the duodenum but, also, to the distal ileum and particularly to the lungs, that is, a distal organ localized completely beyond the area supplied by the compromised arterial bed. Both lines of animals produce PTX3 in the organs involved. As expected, the rate of PTX3 production in transgenic animals is many times higher than that in control mice. Typical features of the damaged organs in either group include in particular:

1. Increased vascular permeability resulting in their edema,
2. Increased inflammatory infiltration due mainly to the activity of polymorphonuclear leukocytes (neutrophils),
3. And development of patches of tissue hemorrhage.

In transgenic animals with high levels of PTX3 production, organ damage is more serious:

4. In the duodenum, there is a more pronounced edema of intestinal vili, whose surface is rugged by deep erosions,
5. The extent of cellular infiltration of the intestinal wall is many times larger,
6. The lungs of transgenic mice also show enhanced inflammatory cellularization and larger extent of edema. Unlike control animals, the damage is not confined to the tissue interstitium, with edematous fluid flooding also the inside of most alveoli.

In their involved organs, transgenic animals generate larger amounts of proinflammatory cytokines (TNF- α , IL-1 β) and macrophage chemotactic protein-1 (MCP-1). However, the biggest difference is the many times higher plasma levels of TNF- α noted in transgenic mice. Administration of soluble TNF- α chimeric receptor (sTNFR1-IgG) to transgenic mice immediately prior to the start of reperfusion decreases their mortality to that seen in control animals (33). There is a positive feedback between PTX3 and TNF- α . PTX3 production is induced by TNF- α , but there is also an opposite association, with TNF- α production being further increased by the activity of PTX3. This reciprocity also occurs in the systemic inflammatory reaction. Provided the reaction has been

induced by microbial agents, the resulting activity of PTX3+TNF- α is beneficial for the body. In the event of a sterile inflammation without a clearly defined target, the tandem of PTX3+TNF- α has a devastating impact on the body.

PTX3 DEFICIENCY: A PROBLEM OF IMMUNOCOMPROMISED INDIVIDUALS

PTX3-deficient mice ("PTX3 -/-", "PTX3-null") succumb to infection caused by the fungus *Aspergillus fumigatus*. Wild type mice are resistant to the infection and most of them survive it. Invasion of aspergillid conidia (spores) into terminal airways is followed by production of proinflammatory cytokines in the strictly coordinated sequence IL-18 \rightarrow TNF- α \rightarrow IL-12 \rightarrow IFN- γ . As increased IL-18 production does not require enhanced mRNA transcription (rise in the production of this cytokine occurs via increased cleavage of the precursor molecule pro-IL-18 by an enzyme called caspase-1), TNF- α becomes the key molecule for the generation of the subsequent proinflammatory cytokines and, hence, the one providing for the defense of the invaded body against aspergillid infection in a decisive manner (34, 35).

In PTX3 -/- mice with inadequate TNF- α production, the course of infection is typically associated with massive colonization of the lungs and brain by the fungal pathogen (36). Defenses against *Aspergillus fumigatus* are provided both by phagocytosing macrophages and by activation of Th1-lymphocyte response, i. e., antigen-specific immune response mediated by cytotoxic T-lymphocytes (37). The lungs of infected PTX3 -/- mice show decreases in the levels of IFN- γ , a macrophage-activating cytokine, and IL-12, the cytokine polarizing the Th1-lymphocyte response. By contrast, there is an increase in the local levels of IL-4 and IL-10, i. e., cytokines with immunosuppressive action. On contact with aspergillid spores, dendritic cells of PTX3 -/- mice are unable to produce IL-12 or raise MHC class II production. The result is inadequate bactericidal efficacy of alveolar macrophages and inadequate development of the Th1-lymphocyte response (38). This leads to a relative predominance of the Th2 response, i. e., the antigen-specific immune response mediated by antibodies (immunoglobulins) (39). However, antibodies are not of key relevance for effective defense against aspergillid infection (40). In contrast, mononuclear phagocytes of wild type mice or healthy man exposed to the action of conidia of the mold *Aspergillus fumigatus*, increase PTX3 production on a regular basis. PTX3 facilitates phagocytosis of aspergillid conidia by alveolar macrophages with subsequent intracellular killing of these conidia. The role of PTX3 in the control of aspergillid infection is further underlined by the fact that administration of exogenous PTX3 decreases the mortality of infected PTX3 -/- mice to rates observed in control animals. Complement factor C1q-deficient mice are likewise susceptible to infection caused by the above conditional pathogen. Exogenous PTX3 also confers effective protection to C1q -/- animals. On the other hand, no difference has been documented between PTX3 -/- and normal animals in their ability to resist infection caused by bacterium *Listeria monocytogenes*. The defense of PTX3 -/- individuals is not compromised in this case. PTX3 thus plays a crucial role at least in inborn resistance against the fungal pathogen *Aspergillus fumigatus*, but isolated PTX3 deficiency does not entail a general decrease in body's defense (41). In the future, PTX3 could potentially enhance the defense of systemically immunocompromised individuals, such as HIV-positive individuals or bone transplant recipients. Administration of exogenous PTX3 to mice undergoing allogeneic bone marrow transplantation and inoculated *Aspergillus fumigatus* conidia will confer protection against the development of lethal infection. In this indication, the efficacy of PTX3 is comparable or even superior to that of amphotericin B (42).

Abbreviations

- APCs - antigen-presenting cells
- CLP - caecal ligature and puncture
- CRP - C-reactive protein
- DNA - deoxyribonucleic acid
- IFN - interferon
- IL - interleukin
- L - ligand
- MCP - macrophage chemotactic protein
- PTX3 - pentraxin 3
- SAP - serum amyloid P
- TCR - T-lymphocyte receptor
- TGF - transforming growth factor
- TNF - tumor necrosis factor
- TSG-14 - TNF-stimulated gene 14

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More detailed information of the reviewed topic including numerous excellent illustrations is available in: **Maruna, P.:** *Proteiny akutní fáze. Fyziologie, diagnostika, klinika.* Praha, Maxdorf, 2004, 282 p.

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Translation: René Prah

COMMENTARY

Comments on the article by P. Kuneš “Pentraxin 3 in the inflammatory and immune response”

Pentraxin 3 is a protein belonging to the class of so-called long pentraxins - proteins involved in the control of inflammatory processes and produced mainly under the influence of proinflammatory and anti-inflammatory cytokines. Unlike the known and in clinical practice routinely used C-reactive protein belonging to the so-called short pentraxins, pentraxin 3 is the best to date characterized representative of the group of so-called long pentraxins.

The role of this protein in the inflammatory and immune responses, particularly in connection with exogenous infections and, partly, in immune system activation of other etiology, is described in detail in the review article by Pavel Kuneš. Just as with other substances identified in recent years, essential knowledge about the function of pentraxin 3 has been obtained by research in transgenic mice with enhanced production of the protein or, conversely, in animals with completely inhibited pentraxin 3 synthesis by genetic manipulation (in so called PTX3-knockout mice).

Mice with increased PTX3 production feature increased resistance to lipopolysaccharide-induced inflammatory stimulation and, also, to the model of bacterial peritonitis induced by ceacal ligation and puncture. This resistance is primarily due to the more rapid rise in the levels of tumor necrosis factor- α , leading to faster and more intensive mobilization of granulocytes, tissue macrophages and fibroblasts. However, in an experimental model of sterile inflammation—caecal reperfusion following temporary arterial mesenteric superior occlusion—PTX3 overproduction with subsequent excessive increase in the levels of tumor necrosis factor- α is detrimental and accelerates tissue damage.

PTX3 deficiency leads to an impaired inflammatory reaction resulting in markedly reduced resistance of PTX3-knockout mice to infection caused by *Aspergillus fumigatus*. Whilst healthy animals are relatively highly resistant to this infection, PTX3-knockout mice show inadequate immune system activation primarily due to decreased tumor necrosis factor- α release. The result is an impaired activation cytokine chain with reduced production of the proinflammatory agents interferon- γ and interleukin-12 and increased production of anti-inflammatory cytokines.

In addition to a number of papers addressing other aspects of PTX3 involvement in “conventional” immunological processes, there have been papers dealing with the currently “hot” and most attractive issue regarding the relationship of systemic inflammatory reaction to atherosclerosis and cardiovascular disease. Though not the topic discussed in the review article, there is no doubt it should likewise be mentioned.

At present, it is taken for granted that increased immune system activation (most often in the form of so-called subclinical inflammation) plays an important etiopathogenic role in the onset and development of not only atherosclerosis but, also, insulin resistance and type-2 diabetes mellitus. Data from large epidemiological studies have shown these processes are closely inter-related in a cluster of diseases referred to as the metabolic or Reaven’s syndrome, associating visceral obesity, insulin resistance, dyslipidemia, arterial hypertension and a number of other abnormalities. It is also a well known fact that the metabolic syndrome will raise (up to several times) the risk for cardiovascular complications compared with populations free of the syndrome.

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What is the association between pentraxins and the metabolic syndrome? The best characterized pentraxin, i. e., C-reactive protein, has seen its premiere as a marker of subclinical inflammation and has already been in clinical use as a marker of subclinical inflammation in patients with atherosclerosis (the technique is one so-called high-sensitivity CRP determination, as an increase in levels in these patients is below the detectability limit of conventional determination of this agent used in inflammation). A number of papers have recently been published demonstrating that PTX3 determination in this indication (and/or in other cardiovascular indications) may be superior to CRP determination.

For example, as early as 2000, PTX3 levels were demonstrated to rise in acute myocardial infarction patients. Similarly, in another study, performed in critically ill patients, PTX3 levels correlated with disease severity and were significantly increased compared with those found in a control group of healthy individuals. Most interesting data were furnished by the large study designed by Latini et al. conducted in more than 700 patients with acute myocardial infarction. The study showed PTX3 levels were the best 3-month survival predictor of these patients.

There is also direct evidence regarding PTX3 production in atherosclerotic lesions. For example, Klouche et al. demonstrated that oxidized LDL complexes stimulate PTX3 production in smooth muscle cells. PTX3 production in these cells is significantly inhibited by statins, which may be just another mechanism whereby these drugs modulated atherosclerosis progression. In another completely unique study, PTX3 production was significantly stimulated by resistin—one of the newly discovered hormones derived by adipocytes and macrophages, assumed to have an etiopathogenic contribution in the onset and development of atherosclerosis and insuline resistance .

Pentraxin 3 is then another protein with a major role in immune processes, whose additional roles in the human body we are just beginning to recognize. Future studies will no doubt show whether or not this protein is a better prognostic marker of atherosclerosis and whether or not its determination has clinical relevance more extensive than with selected infectious or autoimmune diseases.

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Translation: René Prahľ

COMMENTARY

The article by P. Kuneš “Pentraxin 3 in the inflammatory and immune response”

The article provides an excellent overview of currently available data about the role of the multifunctional recognizing protein in the complex mechanism of the body's immune defense, data on a function, which has by far not yet been completely understood and is still being explored by an army of experts of various specialties. This feature makes the review article a most timely publication. For reasons easy to understand, the author strictly keeps to the topic chosen with short references putting the issues into a broader context. It was perhaps this approach which made me reflect on the rapid development of the study and disputable concept of a class of substances called lectins.

Pentraxin 3 (PTX3) was the first “long” pentraxin (1) to be identified 13 years ago and, given to structural and functional analogies, it was included, along with the pentraxins, to the most heterogeneous group of proteins called lectins (from Latin *legere* = select, collect). Their ability to specifically recognize branched oligosaccharide groups of glycoproteins and glycolipids was believed to make the basis of their common feature to bind to target molecules and cells (2). Lectins “read” the branched structure of carbohydrates as if in the Chinese sign alphabet, a system of transmission of information qualitatively different from the linearly structured language of nucleic acids (3). This

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phylogenetically very old quality has selected lectins as molecules suitable for a host of functions in organisms, plants, and animals. With their recognition potential, animal lectins serve both outside the immune system (e. g., they are involved in protein distribution within the cell and in determining their viability, in monitoring proper folding of newly synthesized proteins) and inside it, to recognize molecules and cells of the immune system. We are familiar with lectins' direct protective function similar to the role of antibodies (immunoglobulins are not classified as lectins), their specific interactions in controlling migration of cells (selectins), in modulating immune reactions, and in preventing autoimmunity.

However, research in recent years has furnished information extending and complicating the definition of lectins. Similarities have been found among lectins, which were not due exclusively to mutations of the original gene of related lectins (evolutionary divergence) but, also, due to evolutionary convergence whereby lectins have attained—via several independent lines of evolution—to similar spatial folding and to similar recognition (binding) properties despite their different primary structure (amino acid sequence) (3). The target structures (ligands) of some lectins including pentraxins are not only oligosaccharide groups but, also phospholipids, peptidic structures and DNA or histones (4, 5). The long pentraxin PTX3 which, despite some structural and functional analogies with short pentraxins (CRP and SPA), does not use calcium ions for ligand binding, does not recognize oligosaccharides, phosphocholine or phosphoethanol amine whilst binding specifically to the structure of the C1_q component complement as do CRP and SPA (6). Definition of lectins is likely to pose an increasing challenge and even might be useless as, after all, suggested by the absence of this term in Kuneš's review article.

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Translation: René Prahľ

Takotsubo cardiomyopathy: A case report and overview of the relevant literature

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SUMMARY

The authors present an interesting case report of 69-year-old Caucasian woman with Takotsubo cardiomyopathy. Takotsubo cardiomyopathy is a relatively recently characterized heart syndrome that probably develops due to the direct toxic effect of excessive release of catecholamines on cardiac adrenoreceptors during emotional or physical stress. Its typical features include reversible left ventricular apical dyskinesia, chest pain with ST-T changes on the ECG, minimal myocardial enzymatic release and the absence of coronary stenosis on the coronary angiogram. Early coronary angiographic examination is highly recommended as the clinical picture of this syndrome mimics acute myocardial infarction. Beta-blockers are considered to be the treatment of choice.

Key words: cardiomyopathy, apical left ventricular ballooning, echocardiography.

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CASE REPORT

A 69-year-old heavy female smoker (40 cigarettes a day) of Caucasian race, with arterial hypertension, on long-term follow-up at the Outpatient Lung Unit of our department for chronic obstructive pulmonary disease (COPD), was admitted in early June 2004 for one-week lasting intermittent pressure pain below the lower edge of the sternum, experienced on minimal exercise. A week prior to the onset of these problems, the patient had her dose of oral corticosteroids increased for bronchial obstruction deterioration. However, her exertional dyspnoea persisted even after the change in medication. An additional important fact in the patient's history was increased psychological stress due to social insecurity for several weeks.

On admission, the patient was eupnoic at rest, free of cyanosis, filling of her jugular veins was not increased, lung percussion produced full, clear resonance alveolar breathing with diffuse expiratory wheezing, with audible systolic murmur of intensity 2/6 over the cardiac apex, cardiac action was regular at 60 beats/minute, blood pressure 120/80 mmHg. The ECG documented sinus rhythm with normal conduction intervals; there was evidence of 1-mm ST-segment elevations in leads II, III, aVF, V4, V5, and, partly, also in V6 associated with clear T-wave negativity (Fig. 1).

Based on ECG finding, the patient immediately underwent transthoracic echocardiography, documenting an extensive area of dyskinesia in the apical region of non-enlarged left ventricle (LV) and extending over the apical half of all LV walls (Fig. 2). An addi-

tional finding was hyperkinesis of LV basal segments; the resulting LV ejection fraction (LVEF) was decreased, being 35%. The LV walls were not hypertrophied. In the LV outflow tract, there was evident dynamic subaortic obstruction due to the systolic anterior movement of the mitral valve anterior cusp with maximal pressure gradient of 39 mmHg (Fig. 3). Mitral regurgitation of intermediate significance, directed eccentrically under the posterior cusp of the valve, was present as a result of impaired coaptation of mitral leaflets.

The patient immediately underwent selective coronary angiography with a normal finding on the epicardial coronary arteries.

A working hypothesis of Takotsubo cardiomyopathy was established based on the patient's history and results of ECG, echocardiography and coronary angiography. The patient was placed on a monitored bed of the coronary care unit. In addition to her chronic medication including oral theophylline, methylprednisolone and N-acetylcysteine and inhaled budesonide and ipratropium, therapy was initiated with a beta-blocker, initially 10 mg metoprolol i. v. in two divided doses at 30-minute interval, with subsequent switchover to oral therapy at a dose of 25 mg b.i.d.. Therapy also included oral acetylsalicylic acid, subcutaneous enoxaparin and alprazolam to reduce psychological tension. Laboratory investigations revealed a mild elevation of cardiospecific markers with peak values of MB-creatinine kinase of 0.59 μ kat/l and troponin I of 2.05 μ g/l. Chest x-ray showed no signs of left-heart insufficiency, only the picture of COPD.

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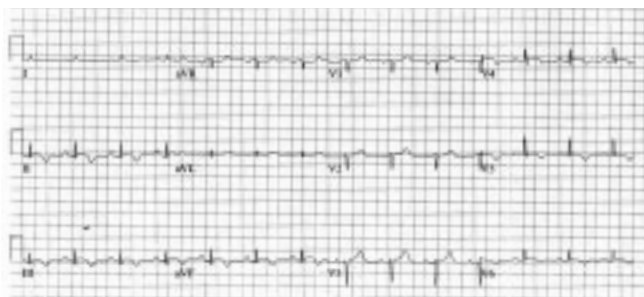


Fig. 1. ECG finding on admission
One-mm ST-segment elevations are present in leads II, III, aVF, V4, V5, and, partly, in V6 along with T-wave negativity.

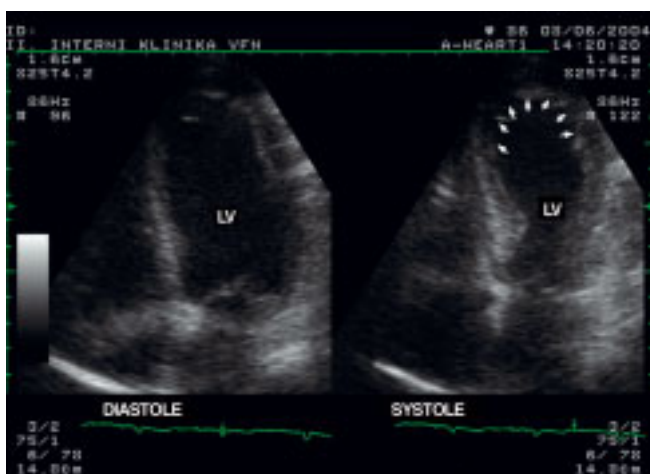


Fig. 2. Echocardiographic finding on the day of admission
The apical four-chamber view shows marked apical dyskinesia (arrows).
LV - left ventricle



Fig.3. Echocardiographic finding on the day of admission
The left panel includes a continuous-wave Doppler recording of accelerated blood flow in the left ventricular outflow tract at the site of dynamic subaortic obstruction with a maximal pressure gradient of 39 mmHg.
LV – left ventricle, LVOT – left ventricular outflow tract

Given her circulatory stability, the patient was transferred, on her second day of hospitalization, to standard-care ward with telemetric monitoring. She continued to receive the above medication plus the angiotensin-converting enzyme inhibitor perindo-

pril at an initial dose of 2 mg daily. Therapy with acetylsalicylic acid and enoxaparin was discontinued. Virology performed to exclude the possibility of myocarditis was negative, as were laboratory markers of inflammation. No significant arrhythmias were registered throughout the hospitalization. Echocardiography performed on day 2 of hospitalization documented improvement of LVEF to 44%. While at rest, the dynamic LV outflow tract obstruction was no longer present, it did reappear immediately during Valsalva maneuver; mitral regurgitation was significantly reduced. Complete remission of the dynamic obstruction was documented by echocardiography after one-week hospitalization, when there was also virtually normal global systolic LV function with LVEF of 55% in the presence of minor area of apical akinesis. Clinically, the patient stopped complaining of chest tightness on exertion already on therapy initiation, and the problem did not recur even after completion of bedrest at five days of hospitalization. Exertional dyspnoe persisted as did the auscultatory finding of bronchitis, whose remission required two-week intensive bronchodilator and expectoratory therapy. On the ECG, there was remission of ST-segment elevations over the first week, followed by T-wave normalization over the next 3 weeks. The patient was discharged to receive home care after 3 weeks of hospitalization, only complaining of mild exertional dyspnoe. Follow-up by echocardiography at one month after the onset of problems demonstrated completely normal kinetics of all LV segments in the presence of normal global systolic LV function with LVEF of 67% (Fig. 4), absence of either resting or provoked dynamic subaortic obstruction (Fig. 5), and minimal mitral regurgitation.

DISCUSSION

Takotsubo cardiomyopathy was first described by Satohe et al. (1) and Dote et al. (2) in the early 1990s. Takotsubo is the Japanese term for a special fisherman's container ("tsubo") used to catch the octopus ("tako"). The shape of the container with a narrow neck and a circular bottom closely resembles to the ventriculogram of the LV in Takotsubo cardiomyopathy. Alternative terms used in the literature include ampulla cardiomyopathy or transient left ventricular apical ballooning. While most cases were originally identified in Japan, there have been reports describing the condition also in individuals of other than Asian origin (3); hence, the disease is not race-related.

Takotsubo cardiomyopathy is characterized by 1) acutely occurring and reversible LV apical impaired kinetics in terms of apical balloon-like dyskinesia, with basal LV segment hypercontractility; 2) chest pain; 3) ECG changes of the ST segment and T-waves, typically ST-segment elevations in leads II, III, aVF and V2-V5; 4) minimal increase in the levels of cardiac-specific markers; and 5) absence of significant coronary stenosis demonstrated by coronary angiography (4). The etiology and pathogenesis of the disease remains poorly understood. Original case reports suggested multiple vasospasms with subsequent development of stunned myocardium (1, 2). A multicentric study by Tsuchihashi et al. included retrospective analysis of 88 patients hospitalized on the basis of clinical problems and ECG finding with suspected acute myocardial infarction, in whom the diagnosis of Takotsubo cardiomyopathy was later established based on the above criteria (5). In Tsuchihashi's paper, provoked vasospasm was only documented in 21% of patients. As a result, multiple epicardial coronary arterial vasospasms do not seem to be the main cause for the development of this type of cardiomyopathy. The intraventricular pressure gradient resulting from dynamic subaortic obstruction was present in 18% of patients. Most patients were women over 60 years of age. A

puzzling fact was the finding that the onset of the disease was preceded, in 70% of patients, by a stressful situation, emotional or physical such as stroke, epileptic seizure, bronchial asthma exacerbation, surgery, or strenuous physical activity. Consequently, psychological and physical stress was identified as the very likely triggers of the whole process. Increased sympathetic activity leads to excessive release of catecholamines acting directly on the myocardium, and resulting in hyperactivation of beta-adrenergic receptors with their subsequent uncoupling and impaired myocardial contractility. The predilection involvement of LV apical area can be explained by several facts (5). The three-layer structure of the myocardium is not present in the ventricular apex, with loss of elasticity readily occurring in the region due to excess expansion. In addition, the cardiac apex is the borderline zone (locus minoris) of areas supplied by major coronary arteries, with the highest density of adrenoceptors (6).

The “catecholamine” theory of development of Takotsubo cardiomyopathy is supported by several other findings. Using radionuclide techniques, Owy et al. compared myocardial perfusion and functional sympathetic innervation in their patients (7). Impaired sympathetic innervation in the apical region was obvious and associated with dyskinesia, while myocardial perfusion was not severely impaired even in acute phases of the disease. Cardiac adrenoceptor activation as the primary cause of this clinical syndrome is also suggested by observations from an animal experiment whereby emotional stress in rats induced transient reversible contraction impairment involving the LV apex, which could be terminated by the administration of amosulolol, an adrenoceptor inhibitor (4). The beneficial effect of beta-blocker therapy has also been reported in man (8). Invaluable data emerged from a study by Abeho et al., who consecutively identified 17 individuals with Takotsubo cardiomyopathy. Focal coronary artery vasospasm was only reported in a single patient; no marked microcirculatory impairment was documented by intracoronary Doppler examination combined with contrast echocardiography (9). Serology and endomyocardial biopsy did not reveal signs of acute myocarditis in any of the patients. Just as in the study by Tsuchihashi et al. (5), emotional or physical stress was identified as the trigger in most patients (94% of individuals).

Intraventricular subaortic dynamic obstruction of the LV outflow tract, present in some individuals (18% in the study of Tsuchihashi et al., 15% in the report of Desmet et al., none in the study by Abe et al.), is currently considered as a secondary factor occurring due to basal LV segments hypercontractility and not as the primary cause of the development of apical dyskinesia. Still, one may speculate about its role in perpetrating apical dyskinesia due to potentiation of high systolic ventricular wall tension (3).

LV apical regional dyskinesia usually resolves within several days to weeks (3). The course of the disease is usually uncomplicated; however, there have been reports of thrombus formation in the LV apex (10), development of cardiogenic shock requiring mechanical circulatory support (11) and fatal ventricular wall rupture (12). Takotsubo cardiomyopathy could thus be another cause of sudden cardiac death; hence the recommendation of close bedside monitoring of these patients in a coronary care unit with regular follow-up by echocardiography.

We believe the case reported by us fully meets the criteria of Takotsubo cardiomyopathy. To the best of our knowledge, this is the first case reported in the Czech relevant literature. In the differential diagnosis of this disease, the clear priority is to rule out acute myocardial infarction, as management of both entities is different and, particularly, administration of thrombolytic therapy to a patient with Takotsubo cardiomyopathy could appreciably raise the risk of LV wall rupture, present in this syndrome (11). It is therefore critical to perform selective coronary angiography to exclude signifi-

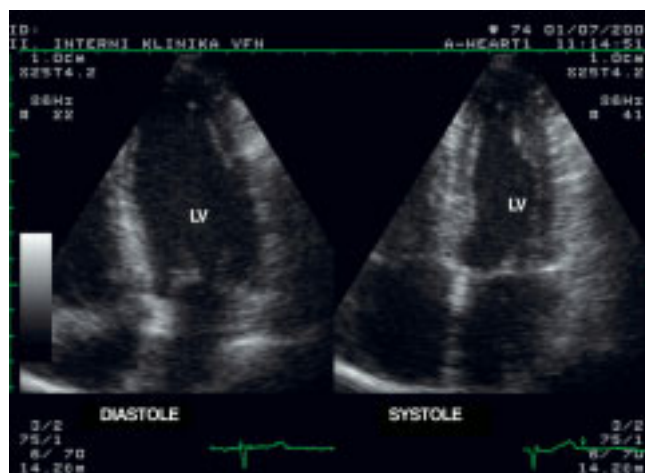


Fig.4. Echocardiographic finding at 1 month
The apical four-chamber view shows normal kinetics of all LV segments.
LV – left ventricle

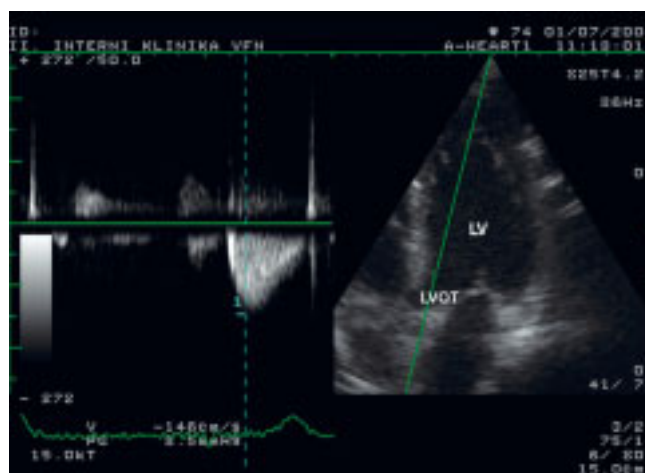


Fig.5. Echocardiographic finding at 1 month
The left panel includes a continuous-wave Doppler recording of blood flow across the left ventricular outflow tract showing normal values of this flow.
LV – left ventricle, LVOT - left ventricular outflow tract

cant coronary artery disease. Of equal importance is echocardiography documenting the typical morphological finding on the LV; alternatively, ventriculography can be performed during catheterization. The drugs of choice, because of the assumed mechanism of onset of the disease, i. e., emotional or physical stress due to myocardial stunning, and because of the beneficial effect documented in the literature, are beta-blockers. In our view, anxiolytics are a class of drugs appropriate in patients with a clearly expressed psychological component. Consistent monitoring of patients, at least over the first days of hospitalization, is imperative considering the potential risks of the disease.

Abbreviations

- COPD - chronic obstructive pulmonary disease
- ECG - electrocardiogram
- EF - ejection fraction
- LV - left ventricle
- LVOT - left ventricular outflow tract

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Translation: René Prahľ

COMMENTARY

Comments on the article by Paleček T. et al. "Takotsubo cardiomyopathy: A case report and overview of the relevant literature"

This is an interesting case report as it is the first description of so called Takotsubo cardiomyopathy (TC) in the Czech literature. The first to characterize this special condition were the Japanese authors Satoh et al. in 1990 (reference in the Paleček's paper). While many of us have seen patients with this disease before, we were unable to classify it and would usually refer to it as a variation of hypertrophic cardiomyopathy.

Today, the disease at least has a name (although somewhat strange and making absolutely no sense to most Europeans); however, our unease and uncertainty over exact classification of TC are no smaller than they were before its identification.

The patient in the case report had a typical TC echocardiographic picture with apical dyskinesis, hyperkinesis of other myocardial regions and, also, the ECG finding (ST-segment elevation in II, III, aVF, and in precordial leads). She was typically an elderly female with chest pain developing after psychological trauma; other features consistent with literary data included the course of the disease with normalization of myocardial kinetics and mild left ventricular outflow tract obstruction.

A role in the onset of TC may be played by genetic factors, as evidenced by more recent reports (1). Coronary spasms could represent a potential pathogenic factor; however, they can only be documented on exceptional cases, and this even after pharmacological provocation. Of interest in this context are no doubt recent data of Nishikawa and coworkers (2) demonstrating a notable decrease in coronary reserve, as measured by intracoronary Doppler ultrasound, which could imply a coronary microcirculation disturbance; however, these results are inconsistent with those of earlier reports included by the authors in their list of references (Abe et al.). Likewise, Nishikawa et al. suggested the possibility of mild right ventricular involvement (3); coronary reserve improved—as did the disease as a whole—after 30 days since onset.

Mild positivity of cardiospecific enzyme is a usual, yet not constant finding. Increased levels of serum noradrenaline are increased on a regular basis (3).

TC can be distinguished from myocardial infarction by only exceptional presence of abnormal Q-waves and absence of "mirror" ST-segment depressions in contralateral leads (4). No doubt we will not dare indicate coronary angiography on the first suspicion of TC, as the degree of overlapping of clinical, electrocardiographic, laboratory, and echocardiographic features is high for both conditions.

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The prognosis of TC in most patients seems to be favorable; BNP determination does not allow identifying those with a potentially unfavorable course (5). Complications of TC may include rupture of the free wall of the left ventricle, formation of intracardiac thrombi and, possibly, severe heart failure suggestive of cardiogenic shock. While beta-blockers are the preferred medication, the condition may resolve even without therapy (3).

Future research into TC will no doubt provide further insights into this particular disease and, perhaps, some surprises. One should expect researchers to focus their attention on the etiopathogenesis of the disease and definition of the molecular-genetic basis of the entity.

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