

**Basic science for clinicians series**

Treating inflammation in atherosclerotic cardiovascular disease: emerging therapies

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Atherosclerosis constitutes the underlying disease to the clinical manifestations of myocardial infarction, stroke, and gangrene. Despite the success of statins, prevention of clinical events of atherosclerosis remains a major challenge in current-day cardiology. Research into the inflammatory nature of atherosclerosis has led to improved mechanistic understanding of its pathogenesis and to the identification of novel therapeutic targets discussed in this review. Recent genetic and epidemiological data document shared pathologies of chronic inflammatory diseases and atherosclerosis. Anti-inflammatory treatment regimens used in these diseases, including tumor necrosis factor- α blockade, IL-1 receptor antagonism, and leukotriene blockade may be beneficial also in patients with coronary artery disease. Enhancing inherent atheroprotective immunity by expansion of regulatory T cells may emerge as a future therapeutic strategy. Immunization strategies directed against atherosclerosis-related antigens such as epitopes within the low-density lipoprotein particle have been extensively studied in animal models and may enter the clinical stage. Success of these novel therapies will be critically dependent on the adequate identification of patients and choice of appropriate clinical endpoints.

Keywords Atherosclerosis • Inflammation • Immunity • Therapies • Acute coronary syndromes

Background

Cardiovascular disease represents the main cause of death and morbidity in the western world and is projected to be the number one killer globally by 2020.¹ Cardiovascular medicine faces a need for effective prevention of myocardial infarction, stroke, and gangrene, which constitute the clinical manifestations of atherosclerosis. Currently, 70% of clinical events cannot be prevented with available drug therapy including statins² and at least 10% of coronary events occur in apparently healthy individuals in the absence of major traditional risk factors.³ Better therapeutic opportunities are also needed to limit the extent of damage inflicted on the heart once acute ischemia has occurred, in order to prevent ensuing congestive heart failure (CHF).

Atherosclerosis is a chronic inflammatory disease: clinical evidence

Recent epidemiological studies demonstrate a significant link between coronary artery disease (CAD) and chronic inflammatory diseases.

An increased incidence of myocardial infarction was found in patients diagnosed with rheumatoid arthritis (RA),⁴ systemic lupus erythematosus,⁵ psoriasis,⁶ and gout.⁷ The discovery of an association between a polymorphism in the promoter of the MHC class II transactivator and increased susceptibility to RA, multiple sclerosis and myocardial infarction⁸ provides evidence for a shared immune-mediated pathophysiology in CAD and chronic inflammatory diseases. Expression of MHC class II molecules is induced by the proinflammatory cytokine interferon- γ on several cell types such as macrophages, endothelial cells, and smooth muscle cells and is a prerequisite for antigen presentation and activation of T cells.

Several other genetic variants with known biological functions in inflammation and atherosclerosis were also reported to be associated with myocardial infarction. The products of these genes are involved in adaptive immunity, such as the tumor necrosis superfamily member OX40 ligand (TNFSF4)⁹ that promotes T cell activation. Mediators of innate immunity comprise interleukin (IL)-1 beta,¹⁰ the cytokine lymphotoxin- α (LTA)¹¹ and components of the leukotriene biosynthesis pathway including arachidonate 5-lipoxygenase (ALOX5), arachidonate 5-lipoxygenase-activating protein (ALOX5AP), and leukotriene A4 hydrolase (LTA4H).^{12–14}

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Research on chronic inflammatory diseases has pinpointed members of the tumor necrosis factor superfamily (TNFSF) (signature cytokine: TNF- α , signature cell-type: T lymphocyte)¹⁵ and the IL-1 family (signature cytokine: IL-1 β , signature cell type: monocyte) as key mediators.¹⁶ Chronic inflammatory diseases are now categorized into autoimmune diseases centred around the TNFSF and autoinflammatory diseases characterized by a clinical response to IL-1 β antagonism, respectively.¹⁶ Prominent examples of autoinflammatory diseases are gout and type 2 diabetes mellitus.¹⁶ Coronary artery disease appears to share features of both, autoimmune and autoinflammatory diseases. In CAD patients presenting with acute myocardial infarction, elevated blood levels of soluble and cell-bound members of the TNFSF (TNF- α , CD40L, LIGHT, RANKL, OPG, TRAIL)^{17–22} as well as from the IL-1 family (IL-1 β , IL-18 and IL-33)^{23–25} were detected and correlated with the subsequent risk of cardiovascular death or CHF. Soluble members of the TNFSF and IL-1 family are also present in stable CAD patients.^{26,27} Several other inflammatory mediators downstream of the TNF and IL-1 superfamilies were shown to contribute in the pathogenesis of atherosclerosis.²⁸ A full list of abbreviations can be found in the online supplementary data.

Clinical settings and anti-inflammatory treatment options in atherosclerosis

The dismal results of clinical trials aimed at inhibiting inflammation during reperfusion injury in a non-selective manner

(steroids)²⁹ or by targeting neutrophil recruitment³⁰ suggest placing the focus on a different time point. Coronary artery disease patients at high risk for future coronary events despite currently available treatments and evidence of vascular inflammation constitute the most promising study population for novel anti-inflammatory therapies with respect to sample size and tolerated side effects, placing post-ACS patients at centre-stage. Indeed, a trial termed Cardiovascular Inflammation Reduction Trial (CIRT) has recently been designed. It will compare the immunosuppressive drug methotrexate at very low dose against placebo in addition to the standard treatment in secondary prevention patients.³¹ In such trials, cardiovascular death should be the prime endpoint to move the field forward, complemented by biomarkers modifiable by anti-inflammatory therapies and vascular imaging for adequate patient identification and therapeutic monitoring. *Figure 1* shows potential therapeutic strategies and time points during the evolution of atherosclerosis. Successful novel therapies will need to target inflammation directly without interfering with the cardiovascular risk profile (i.e. lipids) and have an acceptable safety profile. In light of the pathogenetic similarities between CAD and many autoimmune and autoinflammatory diseases, clinical trials conducted with novel immunomodulatory compounds should include registration of cardiovascular endpoints including traditional risk profiles for *post hoc* analysis. One such example may be the immunomodulatory agent fingolimod (FTY720) for treatment of patients with multiple sclerosis³² as experimental data on the role of FTY720 in atherosclerosis were dependent on the model and experimental conditions used.^{33–35}

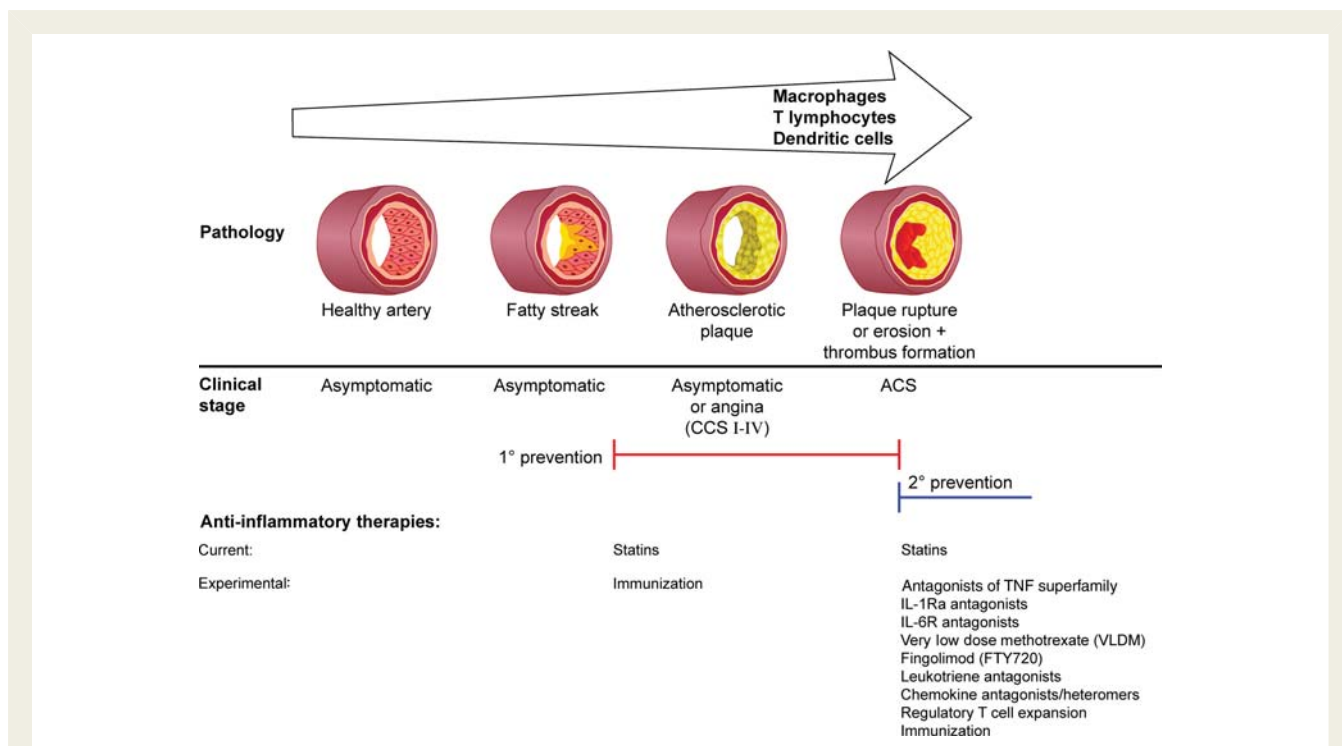


Figure 1 Emerging anti-inflammatory therapies in clinical atherosclerosis. Anti-inflammatory treatment options are shown for the distinct stages in the development of clinical atherosclerosis.

Statins

Statins constitute the best characterized anti-inflammatory class of drugs in primary and secondary prevention of CAD. Beyond their lipid-lowering activity, statins also exert anti-inflammatory effects.³⁶ Clinical evidence for a direct anti-inflammatory effect of statins comes from the *post hoc* C-reactive protein substudies of the PROVE-IT TIMI 22, A to Z, and REVERSAL trials^{37–39} documenting that statin-induced reductions of C-reactive protein and LDL cholesterol levels were only weakly correlated, whereas the decrease in C-reactive protein was significantly correlated with reduced atherosclerosis progression, independent of LDL cholesterol-lowering. The JUPITER trial prospectively confirmed these findings in primary prevention of individuals with elevated C-reactive protein but with low LDL cholesterol.⁴⁰ Further analysis scheduled *a priori* within the JUPITER trial showed that the magnitude of the decrease in C-reactive protein paralleled the magnitude of clinical benefit⁴¹ suggesting a beneficial role of targeting inflammation *per se* in the prevention of cardiovascular events. In addition, the Armyda trial showed that administration of high-dose statins prior to revascularization in ACS patients reduced major adverse cardiovascular events.⁴²

Tumor necrosis factor- α blockade

Tumor necrosis factor blockade has shown efficacy in autoimmune diseases⁴³ and reduced the incidence of cardiovascular events in RA patients, suggesting that attenuated TNF signaling reduces not only RA but also atherosclerosis.⁴⁴ Long-term safety of TNF blockade was documented in RA patients, with no adverse effect on the disease-inherent development of CHF,⁴⁵ adding information to previous reports on CHF patients without RA which showed no adverse effect on hospitalization rates and mortality.⁴⁶ The effects of TNF blockade on plasma lipids require further study.^{47,48} A common feature of TNF antagonists is that they reduce cellularity in inflamed tissues and inhibit expression of pro-inflammatory cytokines and chemokines in addition to TNF- α (IL-1 β , IL-6, IL-8, MCP-1, GM-CSF, VEGF). Furthermore, they dampen the TNF- α -driven production of matrix-degrading enzymes MMP-1 and MMP-3.⁴⁹ These enzymes are considered to be contributing factors to plaque instability.⁵⁰ Tumour necrosis factor antagonism normalized levels of circulating OPG and RANKL in RA patients⁵¹, suggesting an option for therapeutic monitoring. Recent data show that OPG administration can stabilize atherosclerotic plaques in mice.⁵² OPG probably exerts this effect due to its capacity to inhibit RANKL signalling; the latter molecule promotes protease activity and inhibits matrix formation. The pleiotropic cytokine IL-6 acts as a major inducer of the acute-phase response (i.e. C-reactive protein) and impacts on the function of diverse inflammatory and vascular cells. Inhibition of the IL-6 receptor recently shown to be an effective therapeutic in RA patients, however, was associated with elevated lipid levels.⁵³

Interleukin-1 receptor antagonism

Interleukin-1 receptor antagonism (IL-1Ra) has shown beneficial effects in several autoinflammatory diseases¹⁶ and recently also

stroke.⁵⁴ Genetic evidence in a prospective patient cohort documented a significant correlation between a variant in the *IL1-Ra* gene and carotid atherosclerosis.⁵⁵ Experimental data⁵⁶ provided the rationale to conduct clinical trials on the effects of IL-1Ra to prevent post-infarction remodeling (clinicaltrials.gov NCT00789724) and on inflammatory biomarkers in NSTEMI patients, respectively.⁵⁷

Leukotrienes

Leukotrienes belong to the family of eicosanoids and constitute potent pro-inflammatory and smooth muscle constrictive lipid mediators.⁵⁸ Activation of the 5-lipoxygenase pathway in patients with acute myocardial infarction was already documented in 1992.⁵⁹ Recent genetic evidence linked polymorphisms in several enzymes of the leukotriene biosynthesis pathways with myocardial infarction comprising ALOX5, ALOX5AP, and LTA4H.^{12–14} Expression of leukotrienes were detected in atherosclerotic plaques and correlated with symptoms of plaque instability.^{60,61} An ongoing trial aims to evaluate the role of cysteinyl-leukotriene blockade on peripheral endothelial function by administering montelukast to patients after an acute coronary event (clinicaltrials.gov NCT00351364). A recent clinical study showed a marked reduction in inflammatory biomarkers after administration of an inhibitor of the 5-lipoxygenase activating protein (FLAP) to patients carrying at-risk variants in the *FLAP* gene or the *LTA4H* gene.⁶² It remains to be determined whether this tailored therapy translates into a reduction in coronary events.

Leukocyte diversity

The concept of inherent atheroprotective immunity⁶³ has paved the way for therapeutic efforts to attenuate atherosclerosis. Increasing experimental evidence documents a role for several types of leukocytes in atherogenesis, including both pro- and anti-inflammatory subtypes (Figure 2). Circulating monocytes and tissue macrophages can be subdivided into 'inflammatory'/'classical' and 'resident'/'non-classical' subtypes based on the expression of chemokine and adhesion molecule receptors.⁶⁴ These differences may offer possibilities for selectively blocking entry of inflammatory monocytes into atherosclerotic lesions and modify plaque composition. The heterogeneity of T lymphocytes reflects their diverse functions in orchestrating adaptive immune reactions. Four types of CD4+ T helper (Th) cells have currently been identified: Th1, Th2, Th17 cells, and the regulatory T (Treg) cell lineage. Identifying the role of the distinct effector CD4+ T cell subsets in atherosclerosis has been of central interest in recent years.⁶⁵ Recent data identified lymphoid tissue in the adventitia as an additional site to secondary lymphoid tissue (lymph nodes) where naive T helper (Th0) cells may become activated through the process of antigen presentation and co-stimulation.^{66,67}

Expansion of regulatory T cells

A deficiency in Treg cells in terms of number and/or function was shown in patients with a variety of autoimmune diseases that led to the concept that expansion of these cells may attenuate disease activity. Reduced numbers were reported in blood from ACS

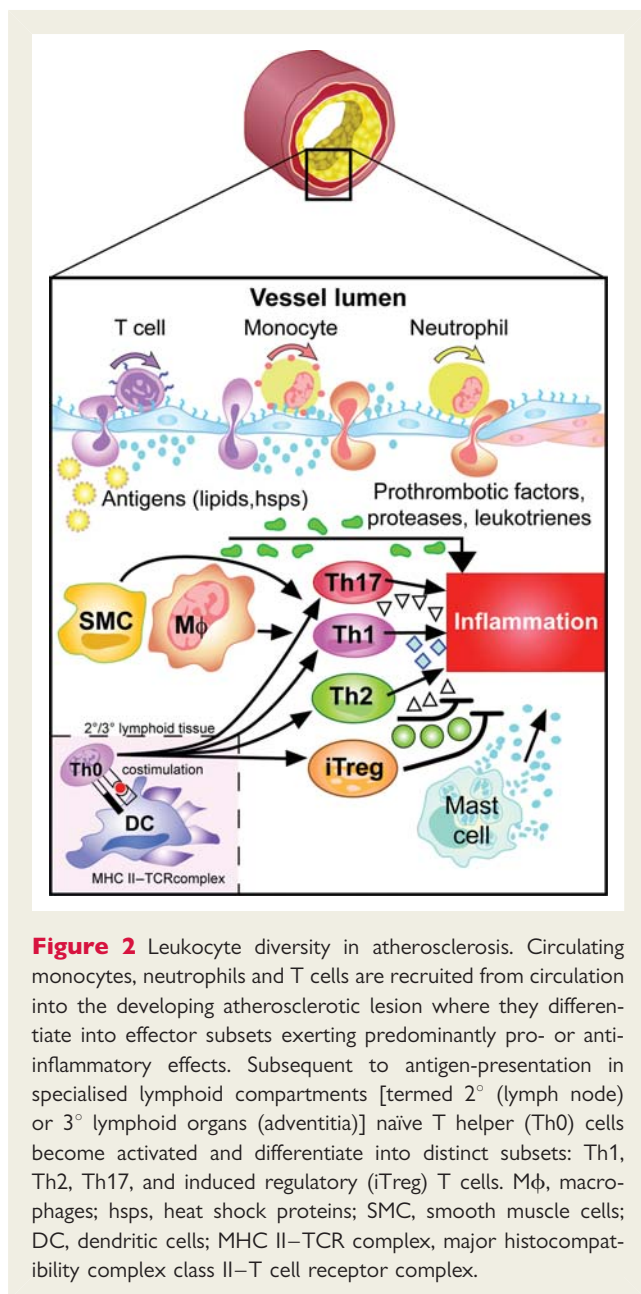


Figure 2 Leukocyte diversity in atherosclerosis. Circulating monocytes, neutrophils and T cells are recruited from circulation into the developing atherosclerotic lesion where they differentiate into effector subsets exerting predominantly pro- or anti-inflammatory effects. Subsequent to antigen-presentation in specialised lymphoid compartments [termed 2° (lymph node) or 3° lymphoid organs (adventitia)] naïve T helper (Th0) cells become activated and differentiate into distinct subsets: Th1, Th2, Th17, and induced regulatory (iTreg) T cells. Mφ, macrophages; hsp, heat shock proteins; SMC, smooth muscle cells; DC, dendritic cells; MHC II–TCR complex, major histocompatibility complex class II–T cell receptor complex.

patients⁶⁸ and Treg cells were detected in all stages of atherosclerotic lesions.⁶⁹ Expansion of the Treg cell pool can be achieved either by promoting Treg cell development and survival *in vivo* by administering drugs or by adoptive transfer of Treg cells following *ex vivo* expansion.

Drugs designed to target surface molecules on T cells selectively deplete activated effector T cells while promoting Treg cell expansion *in vivo*. A monoclonal antibody directed at the CD3-ε chain of T cells showed remarkable efficacy in type 1 diabetic patients⁷⁰ and reduced atherosclerosis in mice.⁷¹ A critical role was recently identified for the co-stimulatory molecules ICOS, PD-1, OX40L, and CD137 in Treg and cytotoxic T cell function in mice,^{72–75} suggesting further targets for modulation of immune homeostasis in atherosclerosis. Targeting the CD40-CD40L pathway appears cumbersome as treatment directed against CD40L produced

thrombosis *in vivo*,⁷⁶ highlighting the need for characterization of the cellular expression pattern and functional aspects of targeting co-stimulatory molecules.

Cytokine administration *in vivo* may be a valid short-term strategy to enhance Treg cells. Interleukin-10 and TGF-β have important roles in Treg cell generation and function. Administration of IL-10 was safe in phase II trials in subjects with psoriasis. TGF-β, however, appears as a less interesting candidate due to its pleiotropic effects.

Adoptive immunotherapy to rapidly increase the circulating Treg cell pool by re-infusion of autologous Treg cells after *in vitro* expansion constitutes an interesting approach. However, several hurdles have to be overcome before translation into the clinics. A pivotal step is the identification and isolation of Treg cells, which poses a major challenge in light of the heterogeneity of Treg cells and the absence of a unifying surface marker. Cell-sorting for CD4+ CD25^{high} CD127^{low} T cells may constitute a valid approach.⁷⁷ In order to provide a disease-specific therapy, re-infusion of antigen-specific Treg cells directed against antigens relevant in atherosclerosis can be achieved by *in vitro* expansion of isolated antigen-specific (adaptive) Treg cells, by means of expanding natural Treg cells isolated from patients against specific antigens *in vitro* or by induced expansion of naïve T cells against specific antigens under tolerable conditions *in vitro*. Several protocols were shown to effectively expand Treg cells *in vitro*.⁷⁸ Introduction of inducible suicide genes allows the control of potential unwanted *in vivo* effects such as leukaemia or generalized immunosuppression upon re-transfer of Treg cells into the host.

Atherosclerosis-specific immunization

Immunization has emerged as a promising therapeutic regimen against atherosclerosis enhancing protective antibody titers, altering the balance of pro- and anti-inflammatory T cell subtypes and expanding Treg cells. Several antigens have been identified and investigated for immunization against atherosclerosis in animal models using active immunization or antibody infusion.⁷⁹ Among those, epitopes recognized in the LDL particle including apolipoprotein B-100 appear most interesting from a clinical perspective in light of the role of LDL in the pathogenesis of atherosclerosis. In order to translate those findings to the clinics, however, antigens that can be easily manufactured under good manufacturing practice conditions and that have a reproducible quality without the risk of contamination are mandatory.

Clearly, more research into the cellular and inflammatory components at different stages of human CAD is needed to identify therapeutic targets, inflammatory biomarkers, and imaging modalities suitable for improved identification of patients and monitoring of anti-inflammatory therapies. Nonetheless, the stage is set to provide a rationale for anti-inflammatory therapies and several clinical studies are currently underway.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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CARDIOVASCULAR FLASHLIGHT

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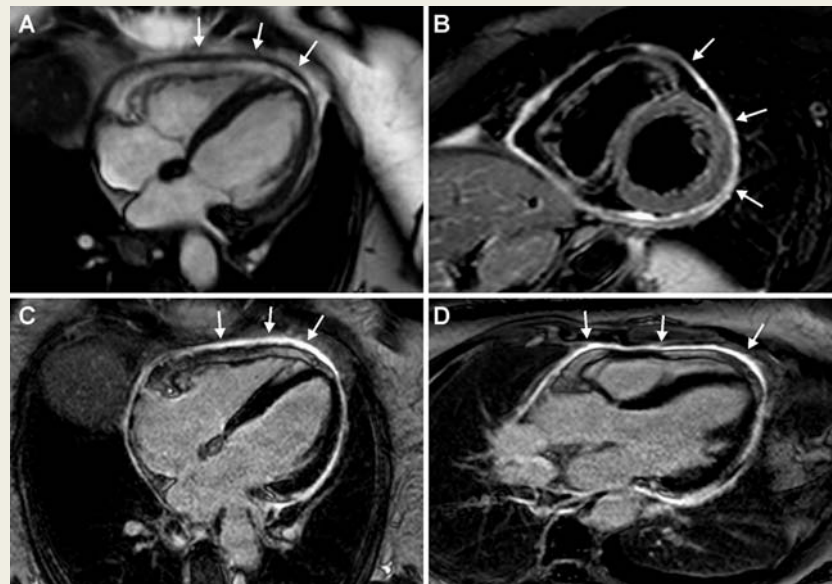
Acute viral pericarditis without typical electrocardiographic changes assessed by cardiac magnetic resonance imaging

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A 59-year-old female was admitted with tachycardia and atypical chest pain. Physical examination was normal. The initial electrocardiogram (ECG) showed atrial fibrillation with a heart rate of 140/min. Laboratory results on admission were unremarkable except for a markedly elevated C-reactive protein of 180 mg/dL (normal value <5 mg/dL). Troponin T levels on admission and follow-up were negative. A follow-up ECG after 4 h documented a spontaneous conversion in sinus rhythm and normal ST-segments. Echocardiography showed a normal left ventricular function (ejection fraction 60%) with no regional wall-motion abnormalities. A small circular pericardial effusion was noted. Additional laboratory tests revealed an acute parvovirus B19 infection by detecting parvovirus B19-specific IgM antibodies in the serum.



Cardiac magnetic resonance (CMR) imaging was scheduled to rule out acute myocarditis. A thickened pericardium (5.5 mm) and a small pericardial effusion were noted at cine-imaging (Panel A). At T2-weighted imaging (Panel B), a hyperintense signal from the thickened pericardium was noted suggesting pericardial oedema. No signs of myocardial oedema were present. Ten minutes after contrast administration of 0.2 mmol/kg gadolinium–DTPA inversion recovery, CMR revealed bright hyperenhancement of the complete pericardium (Panels C and D). No foci of delayed enhancement in the myocardium were noted.

Based on the findings at CMR, acute pericarditis was diagnosed. The patient was treated with non-steroidal anti-inflammatory medication for 4 weeks and remained asymptomatic thereafter.

This case shows that CMR is a valuable non-invasive tool in the differential diagnosis of acute chest pain even in the absence of typical ECG changes. Based on the findings of oedema and contrast enhancement of the pericardium suggesting an acute diffuse inflammatory process of the pericardium, the definite diagnosis of acute pericarditis could be established. Moreover, involvement of the myocardium in the inflammatory process could be excluded.

Panel A. Cardiac magnetic resonance image were obtained with the balanced steady-state free precession technique showing thickened pericardium (5.5 mm, arrows) and a small pericardial effusion.

Panel B. Short-time inversion recovery T2-weighted image show also hyperintense signal from the pericardium (arrows).

Panels C and D. Contrast-enhanced phase-sensitive inversion recovery images [four-chamber view (C) and three-chamber view (D)] in the late phase after gadolinium injection reveal hyperenhancement from the pericardium (arrows).