

# CD 40 SOLUBLE LIGAND AND ACUTE CORONARY SYNDROMES

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Current research is focused on the new cardiac markers that allow detect earlier stages of acute coronary syndromes than is possible with troponins. One of the most promising is CD40 ligand. Clinical data showed that higher levels of sCD40L are associated with a high risk of cardiovascular events. Elevation of soluble CD40 ligand identifies the subgroup of patients who are likely to benefit from antiplatelet treatment with IIb/IIIa antagonists. This marker can be useful for the assessment of risk stratification before planning stent implantation. Future investigation should include large, well-designed prospective observational cohort studies of patients in risk of ACS in order to evaluate the performance of sCD40L as a diagnostic test for ACS.

**Key words:** soluble CD40L, acute coronary syndromes, cardiomarkers.

## SOLUBILNÝ LIGAND CD40 A AKÚTNE KORONÁRNE SYNDRÓMY

Súčasný výskum sa sústreďuje na molekuly, ktorými by sme mohli detekovať skoršie štádiá akútnych koronárnych syndrómov ako sme schopní s troponínmi. Solubilný CD40L predstavuje unikátne spojenie medzi trombózou a lokálnym zápalovým procesom. Podľa klinických údajov sú jeho zvýšené hladiny spojené s vysokým rizikom smrteľného infarktu myokardu, chronického srdcového zlyhania alebo rekurentného infarktu u pacientov s akútnym koronárnym syndrómom. Mohol by odhaliť pacientov, ktorí by profitovali z terapie IIb/IIIa antagonistami a stratifikovať riziko trombózy pred plánovanou implantáciou stentu. K definitívnemu stanovisku sú potrebné ďalšie štúdie.

**Kľúčové slová:** solubilný ligand CD40, akútne koronárne syndrómy, kardiomarkery.

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## Introduction

There is growing interest in the search for new cardiac markers that may allow earlier assessment of acute coronary syndromes (ACS) and superior risk stratification than is currently possible with troponins. Whereas troponins and CK-MB are markers of myocardial necrosis, which is the endpoint of the disease process, research is currently focusing on markers of earlier stages in the pathological evolution of ACS.

One of the most promising is CD40 ligand (CD40L).

## Cellular mechanism

CD40L is a trimeric, transmembrane protein of the tumor necrosis factor family and, together with its receptor CD40, is an important contributor to the inflammatory processes that lead to atherosclerosis and thrombosis<sup>(1)</sup>.

It was originally identified on CD4<sup>+</sup> T cells and later on stimulated mast cells and basophils, recently also has been found on activated platelets. CD40L is also upregulated on platelets in a fresh thrombus<sup>(1, 2, 3)</sup>.

It exists in two basic forms: membrane-bound (mCD40L) and soluble (sCD40L).

Both forms of this ligand may interact with CD40 receptor, which is constitutively expressed on B cells, macrophages, endothelial cells, and vascular smooth muscle cells, resulting in various inflammatory responses, matrix degradation, and also thrombus formation.

Its binding with CD40 leads to the release of procoagulant and atherogenic substances and enzymes which degrade the fibrous cap of atheroma rendering it unstable avoiding the regeneration of endothelial cells.

The CD40L-CD40 interaction seems to play a pathogenic role in a wide range of inflammatory disorders such as autoimmune diseases, multiple sclerosis, graft-versus-host disease, and cardiac allograft rejection. Recently also has been shown to be involved in atherogenesis and plaque destabilization leading to the development of ACS<sup>(6, 7, 8, 9, 10, 11)</sup>.

## Pathophysiology

Although the presence of soluble CD40 ligand reflects platelet activation during a cardiac event, does this immunomodulator itself play a part in the acute thrombotic process?

Traditionally, platelets have been thought of as anuclear, subcellular fragments derived from megakaryocytes circulating in blood as small discs and mitigating hemorrhage.

Haemostatic process requires platelet activation, a complex chain of events involving rapid structural changes that activate adhesion receptors, remodel the cytoskeleton, and lead to the eventual synthesis and secretion of various platelet-derived factors. The result of this cascade is the formation of platelet-dependent thrombosis that primarily attenuates bleeding but, in pathological settings, contributes to occlusive vascular events. Platelets are not only involved in haemostasis but that they also directly initiate an inflammatory response of the vessel wall<sup>(1)</sup>.

The potential additional mechanism for binding between the platelet and monocyte in the setting of acute myocardial infarction is the CD40-CD40L, representing a unique link between thrombosis and inflammation<sup>(12)</sup>.

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The mechanism of the sCD40L-induced cascade of platelet activation is not clearly known:

Platelets express CD40L within seconds after activation *in vitro* and in the process of thrombus formation *in vivo*. Like TNF- and interleukin-1, CD40L on platelets induces endothelial cells to secrete chemokines and to express adhesion molecules, thereby generating signals for the recruitment and extravasation of leukocytes at the site of injury. On endothelial cells or monocytes, the engagement of CD40 with CD40 ligand leads to the synthesis of adhesion molecules, chemokines, and tissue factor and causes activation of the matrix metalloproteinases that are known to contribute to atherothrombotic pathophysiological changes. Soluble CD40 ligand has a lysine-arginine-glutamic acid sequence that allows it to bind to platelet glycoprotein IIb/IIIa. It is possible that such binding is blocked when glycoprotein IIb/IIIa inhibitors are present, potentially altering the clot-stabilizing properties of sCD40L<sup>(13)</sup>.

## Clinical results

The plasma concentration of sCD40L is lower than 5.0 µg per liter in the steady state.

Clinical data showed that levels above 5.0 µg per liter are associated with a high risk of cardiovascular events – sCD40L independently predicts the composite of death/myocardial infarction(MI)/chronical heart failure and particularly of recurrent MI in patients with ACS<sup>(10)</sup>. Notably, simultaneous assessment of sCD40L and TnI, the current standard for recurrent MI prediction, yields independent and complementary prognostic information, thus enabling more powerful prediction of adverse cardiac outcomes<sup>(20)</sup>.

Clinically relevant concentration of sCD40L increased the expression of its receptor CD40 in human coronary artery endothelial cells (HCAECs). The CD40L-induced upregulation of CD40 may be mediated by oxidative stress and extracellular signal-regulated kinase 1/2 (ERK1/2). The sCD40L could enhance its biologic functions in the vascular system and contribute to endothelial dysfunction and vascular disease<sup>(14)</sup>.

Elevated plasma concentrations of soluble ligand CD40 at baseline predict a significantly increased risk for future cardiovascular events in apparently healthy women<sup>(15)</sup>.

Furthermore, patients with unstable angina have higher plasma concentrations of sCD40L than healthy volunteers or those with stable angina<sup>(6)</sup>.

Soluble CD40L plasma levels, however, did not correlate with those of CRP or cTnI<sup>(15)</sup>.

Also in the CAPTURE study soluble CD40 ligand did not correlate with markers of inflammation, including C-reactive protein<sup>(10)</sup>.

Although it has been shown that increased levels of C-reactive protein identify persons at risk for cardiovascular events, C-reactive protein may be a more general marker of inflammation and atherogenesis than soluble CD40 ligand and may be less reflective of the acute thrombotic process<sup>(17)</sup>.

Although platelet-monocyte aggregates can provide useful information about the thrombotic or inflammatory state and can identify patients at high risk for cardiac events, their measurement can be difficult. As compared with platelet-monocyte aggregates, measurement of sCD40L (also called CD154) does not require flow cytometry and can be accomplished with stored samples<sup>(16)</sup>.

Increased sCD40L before percutaneous coronary intervention may increase the rate of stent restenosis. Increased preprocedural sCD40L level is an independent predictor of stent restenosis. This marker can be useful for the assessment of risk stratification before planning stent implantation<sup>(21)</sup>.

## Possible medical impact

Although sCD40L has been characterized as a marker of thrombotic diseases, much less is known about its direct role in platelet function. It has been

suggested that CD40L is an IIb/3 ligand, a platelet agonist, and contributes to stability of arterial thrombi.

The GP IIb/IIIa antagonists inhibit release of sCD40L from activated platelets<sup>(13)</sup>.

Furman et al. examined the effects of glycoprotein (GP) IIb/IIIa antagonists (abciximab, eptifibatide, and tirofiban) and other inhibitors on translocation of CD40L from intraplatelet stores to the platelet surface and on the release of soluble CD40L from platelets<sup>(18)</sup>.

Statins, glitazones, glycoprotein IIb/IIIa inhibitors, and clopidogrel have been demonstrated to effectively reduce CD40L levels both *in vitro* and *in vivo*. Abciximab has been shown to reduce the occurrence of death or myocardial infarction during six months of follow-up in patients with ACS who had the highest levels of sCD40L<sup>(18)</sup>.

Release of sCD40L from platelets is regulated, at least in part, by GP IIb/IIIa. In addition to their well-characterized inhibition of platelet aggregation, GP IIb/IIIa antagonists may obviate the proinflammatory and prothrombotic effects of sCD40L.

In patients with unstable coronary artery disease, elevation of soluble CD40 ligand levels indicated an increased risk of cardiovascular events generally. Elevation of soluble CD40 ligand identifies the subgroup of patients at high risk who are likely to benefit from antiplatelet treatment with abciximab. The prognostic value of this marker was validated in the patients with chest pain, among whom elevated soluble CD40 ligand levels identified those with acute coronary syndromes who were at high risk for death or nonfatal myocardial infarction. The increased risk in patients with elevated soluble CD40 ligand levels was significantly reduced by treatment with abciximab whereas there was no significant treatment effect of abciximab in patients with low levels of soluble CD40 ligand<sup>(10)</sup>.

Also statins, beyond lipid lowering, have pleiotropic effects with favorable benefits against atherogenesis. Withdrawal of statin therapy has been demonstrated to abrogate vascular protective activity and even increase the incidence of thrombotic vascular events. The effect of statin on sCD40L level was abrogated after therapy withdrawal, and was independent of serum cholesterol level<sup>(19)</sup>.

## Conclusion

So far we don't know whether the presence of sCD40L is only an epiphenomenal reflection of the inflammatory and thrombotic processes, or whether it directly contributes to acute coronary damage, but we possess encouraging data that may assist in defining unstable coronary syndromes. Although this marker needs further evaluation to establish its reliability in specific patient populations and clinical settings, this molecule may be an indicator of platelet activation and unstable plaque that can provide information in addition to the commonly used markers of cardiac necrosis.

Soluble CD40 ligand shows promise as an early marker for acute coronary syndromes but large, well-designed prospective observational cohort studies are necessary before it can be implemented as a diagnostic test. Most probably, there can be no single, perfect marker of the complex pathophysiological events contributing to unstable coronary syndromes, but it is possible that a combination of markers that reflect inflammation and thrombosis, before the onset of myocardial damage, would have considerable prognostic and therapeutic value. Future investigation should include large, well-designed prospective observational cohort studies of patients in risk of ACS in order to evaluate the performance of sCD40L as a diagnostic test for ACS.

Incorporation into a "multimarker strategy" may be more likely to yield positive results. However, there is insufficient information to recommend its implementation as a diagnostic test at this time.

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