An Insight about the Suppression of Tumorigenicity 2 Protein (ST2) as Marker of Pediatric Heart Diseases

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Conflict of interest: None declared
Funding: No funding sources

Abstract

The Suppression of Tumorigenicity 2 protein (ST2) is a member of the interleukin (IL) 1 receptor family with transmembrane (ST2L) and soluble (sST2) isoforms both are (over) expressed in various cells in different conditions and following numerous triggers such as inflammation or stress. The Suppression of Tumorigenicity 2 protein (ST2) is one of the most promising biomarkers, with an important role in numerous diseases. Twenty years ago, ST2 was first recognised as an essential factor of cell proliferation, with an influence on cancer development . Later discovery of its inflammatory and immunomodulatory action led to connection with autoimmune and other inflammatory diseases. Increased cardiac loads (increased biomechanical stress, pressure and tension of the muscular structures) due to acute or progressive heart failure (HF), myocytes, vascular structures (endothelial cells) and fibroblasts of the heart increase the expression, formation and release of both forms of ST2. In line with the growing evidence for sST2′s role in the pathophysiological mechanisms of myocardial fibrosis and heart remodelling, several clinical studies have confirmed its potential role in the management of cardiac diseases, especially of HF.
Keywords: The Suppression of Tumorigenicity 2 protein (ST2), Heart Diseases

Tob Regul Sci. ™ 2023;9(1): 5307-5315
DOI: doi.org/10.18001/TRS.9.1.369

Introduction

The Suppression of Tumorigenicity 2 protein (ST2) is a member of the interleukin (IL) 1 receptor family with transmembrane (ST2L) and soluble (sST2) isoforms both are (over) expressed in various cells in different conditions and following numerous triggers such as inflammation or stress **(1).**

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recognised as an essential factor of cell proliferation, with an influence on cancer development . Later discovery of its inflammatory and immunomodulatory action led to connection with autoimmune and other inflammatory diseases **(2)**

Since then, the applications of ST2 have grown continuously. It has been found to be relevant in pulmonary diseases, sepsis and gastrointestinal diseases and is becoming one of the important markers in the management of heart failure (HF), for the indication of disease development and as a prognostic marker for different heart failure populations . ST2 also has a growing potential in tailoring and monitoring therapy of HF patients **(3).**

Recently, its role was confirmed in Chronic Kidney Disease (CKD), especially in End Stage Renal Disease (ESRD). Its pathophysiological characteristics and independence of renal function, give added prognostic value to already established prognostic markers and allow improved identification of patients at high risk for hospitalisation and both cardiovascular (CV) and all-cause death **(1).**

Biology and structure of ST2

The ST2 gene encodes a protein that showed similarities with the immunoglobulin gene superfamily, especially with the interleukin (IL) -1 receptor . ST2 is a member of the interleukin-1 receptor-like-1 (IL1RL1) family and the gene is located on human chromosome 2q12 **(4).**

Following alternative promoter splicing and 3′ post-transcription processing of ST2 mRNA, four protein isoforms of ST2 are produced. The two essential forms are a transmembrane form, ST2 ligand (ST2L) with molecular weight 67 kDa and three extracellular IgG domains, and a smaller secreted soluble form (sST2) with molecular weight 37 kDa, which has a similar extracellular structure but without the transmembrane and intracellular parts. Both molecules act as receptors that bind IL-33 and not IL-1α, IL-1β or IL-1R antagonists and therefore have an essential immunomodulatory function. Additionally, binding of IL-33 to ST2L results in activation of mitogen activated protein kinase (MAPK) and nuclear factor (NF-kB) signalling with various consequent effects in target cells. IL-33 binding with sST2 does not allow and blocks these effects **(5).**

ST2 molecules are expressed as a result of activation and response of the ST2 gene to stimulation by many inflammatory and cell growth factors (platelet-derived, acidic fibroblast and primary fibroblast growth factor), tissue plasminogen activator and the proinflammatory cytokines , like tumour necrosis factor-alpha (TNFα) and IL-1 **(4).**

This expression allows the synthesis of the ST2 receptor molecule in a variety of cells, such as embryonic , hematopoietic, tumour and immune cells, as it belongs to the Toll-like receptor (TLR) family **(5)** . It is present and expressed in fibroblasts and myocytes in the heart muscle **(6).**

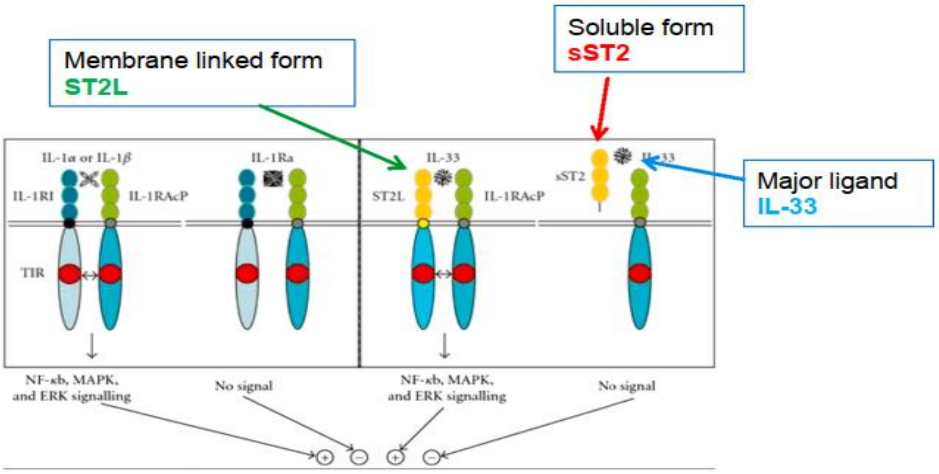

Fig. (1) The structure of ST2L and sST2 as the IL-33 receptor. (IL-1RAcP, interleukin-1 receptor supplemental protein; MAPK, mitogen-activated protein kinase; NFkB, nuclear factor kB) (1).

ST2 as an immune response modulator

The presence and expression of ST2L were initially detected in the T-lymphocytic cell line (LyT), macrophages, in germ cells of the bone marrow and primary mast cells. It is also expressed in dendritic, natural killer cells and activated polymorphonuclear leucocytes (PMNL). Therefore, one of the first ST2 effects was described in association with the immune response in allergic and autoimmune diseases . Various studies confirmed that the role of ST2L in LyT is crucial for the activation and development of T helper type 2 lymphocytes (Th2) responsible for the development of allergic reactions and the production of cytokines IL-4, IL-5 and IL-13 **(7).**

This effect could be avoided by blocking the transmission of signals through the ST2L receptor with the help of antibodies that prevented the binding of the corresponding ligands. Additionally, the direct impact on the development and activation of T helper type 1 lymphocytes (Th1) was largely unchanged, which helped to demonstrate the important role of ST2L in the activation and regulation of Th2 cellular immune response **(8).**

IL-33 is a member of a big IL-1 cytokine family that regulates innate and adaptive immune systems to promote inflammatory responses . In contrast to the action of IL-1, which is processed and released by live immune cells in response to infection or other triggers, IL-33 acts as an alarmin against injury-induced stress, pathogens, or cell death by activating local immune cells **(9).**

IL-33 is a cytokine with dual function. The full-length IL-33 form acts as an intracellular gene regulator (transcription factor) in the nucleus. It repress the expression of NF-κB-regulated genes that are necessary for pro-inflammatory signaling. The mature IL-33 form serves as an extracellular cytokine following release when cells sense inflammatory signals or undergo necrosis **(10).**

Once released from damaged cells upon tissue injury or viral infection, full-length IL-33 can be processed by neutrophil-derived proteases into mature IL-33. It can bind to the membrane-

bound ST2L receptor through its cytokine domain and triggers an inflammatory cascade. Although both forms can bind to and signal through their receptor ST2, mature IL-33 has a 10-fold higher affinity and bioactivity than full length IL-33 **(11).**

The next development was elucidating the role of sST2 as IL-33 soluble receptor and blocker of effects in target cells . Specifically, sST2 bound to IL-33 prevents its binding to ST2L in the immune cell (LyT) . In this way, it also inhibits the activation of Th2 cell response and the release of anti-inflammatory cytokines (IL-4, IL-5, IL-10, IL-13), and modulates the response in the direction of Th1 activation, which results in the activation and release of inflammatory cytokines (TNF-α) and inflammation **(7).**

These processes cause various inflammatory and autoimmune diseases . They are thought to be present in heart muscle, which in turn leads to a worsening of the condition **(12).**

The presence and expression of ST2 gene in various tissues and cells (embryonic tissues, tumour cells, immune cells, fibroblasts, vascular endothelial cells, myocytes) also indicate its broader role and influence in a broader spectrum of biological systems . sST2 is widely distributed and is included in various immune responses and mechanisms resulting in different outcomes in different tissues. Therefore its potential pathophysiological role could make it a significant biochemical marker in the monitoring of a range of inflammatory diseases **(7).**

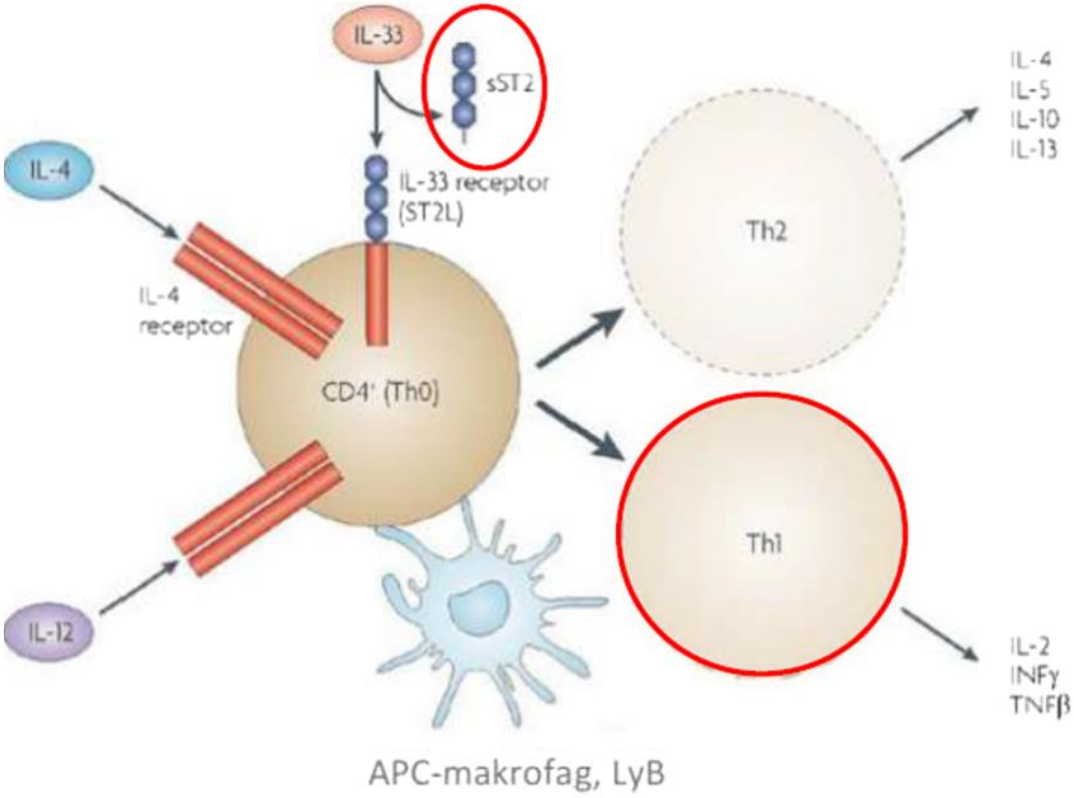

Fig. 2. Effect of sST2 on immune cells: Th2 immune response blocker. Red circles show the mechanism of sST2 action: by blocking IL-33 and preventing binding of IL- 33 to ST2L on CD4 cells, sST2 modifies the immune response/activation and cytokine production from Th2 to inflammatory Th1 response (1).

ST2 and inflammatory bowel diseases

Several studies have confirmed that increased TLR2 expression in the lamina propria is associated with the pathogenesis of inflammatory bowel disease (IBD): ulcerative colitis (UC) and Crohn's disease (CD) **(3).**

Recently, soluble TLR2 (sTLR2) variants have been shown to counteract inflammatory responses driven by the cognate receptor, and increased production of sTLR2 by lamina propria mononuclear cells from ulcerative colitis patients has been confirmed . Further studies have claimed that sST2 can predict the severity and progression of inflammatory intestinal disorders **(13).**

Excellent matching of the serum sST2 concentration with histological findings in patients with IBD (UC and CD), and excellent correlation with faecal calprotectin (an important biochemical marker of the activity of inflammatory processes in the intestine), suggest an important role for sST2 in the monitoring of IBD . sST2 concentrations also correlated well with disease severity and inflammatory cytokines in UC and were able to differentiate active from inactive UC **(3).**

These results indicate a possible role of sST2 as a biomarker in follow up of these patients. Diaz-Jimenez and colleagues have confirmed this role using continuous sST2 measurement to follow changes in the inflammatory activity of UC patients on treatment. They found sST2, like faecal calprotectin, to be a useful biomarker in predicting clinical outcome in UC **(13).**

Source and influence of ST2 on the development of cardiovascular diseases

Increased cardiac loads (increased biomechanical stress, pressure and tension of the muscular structures) due to acute or progressive heart failure (HF), myocytes, vascular structures (endothelial cells) and fibroblasts of the heart increase the expression, formation and release of both forms of ST2 **(14).**

ST2L as the transmembrane receptor is expressed on the surface of myocytes in heart muscle. Binding of IL-33, released from fibroblasts in the heart muscle upon biomechanical stress, high pressure and damage, results in several cardioprotective effects. Several studies have confirmed that binding of IL-33 to ST2L plays a central role in the processes of immune response, homeostasis, and the revitalisation of damaged (heart muscle) tissue. This binding reduces apoptosis of cells exposed to ischemic and inflammatory processes, caused by decreased circulation through the heart muscle tissue or infarction. It also reduces myocardial fibrosis, cardiomyocyte hypertrophy and thus maintains the ventricular function and allows longer patient survival **(14).**

In contrast, the soluble receptor sST2 is simultaneously released from fibroblasts and vascular structures and acts as a blocking receptor by competitively binding to IL-33 and preventing the binding of IL-33 to ST2L and its cardioprotective effects on heart muscle . Therefore, increased expression and release of sST2 enhances

cell apoptosis, the development of fibrosis, hypertrophy, remodelling of the heart muscle and progression of HF **(14).**

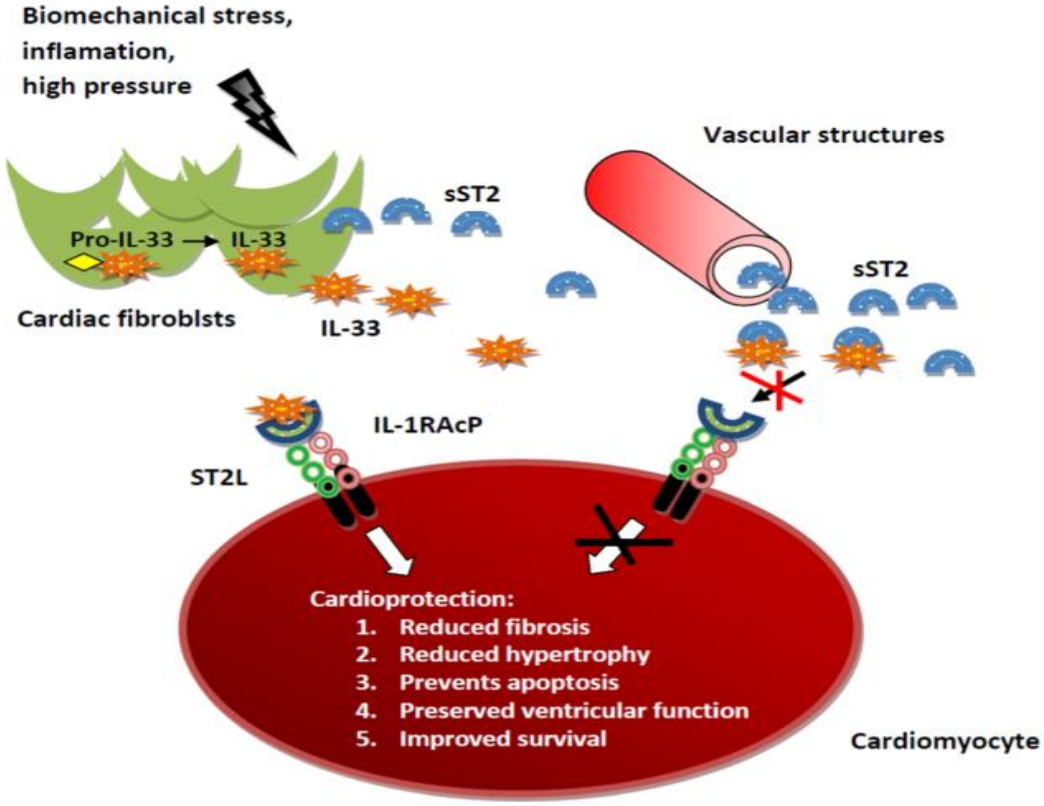

Fig. (3) The effect of IL-33 on binding to the ST2L receptor and sST2 action as its blocker. Sources of sST2 are cardiac fibroblasts and vascular endothelial cells in heart muscle, triggering by biomechanical stress, high pressure and inflammation (1).

sST2 as a marker of heart diseases

In line with the growing evidence for sST2′s role in the pathophysiological mechanisms of myocardial fibrosis and heart remodelling, several clinical studies have confirmed its potential role in the management of cardiac diseases, especially of HF **(15) .**

Natriuretic peptides (NT-proBNP and BNP) are considered to be the gold-standard marker for the diagnosis and management of acute HF.However, in view of the limited prognostic utility of natriuretic peptides and the fact that their role in guiding treatment has not yet been fully established additional biomarkers to fill these gaps are still required and sST2 is an important candidate marker in this respect **(16).**

Several studies have confirmed significant prognostic value for serum sST2 in chronic heart failure in children, where development and prognosis of the disease are strongly correlated with the extent of the fibrosis and remodelling process **(17).**

Several studies suggested that higher levels of soluble ST2 is associated with more adverse events and have robust prognostic value in children with dilated cardiomyopathy and children with complex congenital heart diseases **(18).**

sST2 characteristics

For sST2 to be used effectively in clinical practice it is essential to know the factors influencing measured serum concentrations. These effects may be physiological or the result of pathophysiological processes.Numerous studies have shown that the measured concentrations of sST2 are independent of age, sex, and body mass index **(19).**

unlike natriuretic peptides and galectin-3, sST2 values are not significantly influenced by renal function and are independent of eGFR. sST2 concentrations also correlate well with inflammatory parameters such as CRP, which is consistent with its immunomodulatory role and its influence on the development of the inflammatory process **(20).**

Biological variability:

For serial measurements in patients with chronic disease, biomarkers must have low biological variability (BV), and minimal on circadian rhythm **(21).**

In the absence of significant clinical instability of the study population, IT has been found that the reference change value (RCV) for sST2 (30%) was much lower than RCVs observed with galectin-3 (60%) or NT-proBNP (92%). The calculated index of variation (influence of BV on measured results) was also much lower for sST2 (0.25) compared to galectin-3 (1.0) and showed that sST2 (unlike galectin-3) would be an appropriate biomarker for serial measurement, and therefore suitable for guiding therapy **(19).**

Stability:

Stability of the biomarker is crucial for relevant, comparable and reproducible results both in routine determination and for long-term research studies, in which samples need to be frozen and stored for periods of months or years before analysis. Recently, Dieplinger and colleagues have demonstrated that sST2 is stable in plasma samples stored at -20 °C and -80 °C for at least 18 months, without relevant loss of residual immunoreactivity. The authors acknowledge that their findings may be restricted to the PresageTM ST2 assay, which is currently the only FDA approved and CE marked assay for routine use **(22).**

No Conflict of interest.

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