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MATERNAL CARDIOVASCULAR AND INFLAMMATORY DYSFUNCTION IN **TWIN-TWIN TRANSFUSION SYNDROME**



Clinical Insights from H-FABP, Midkine, sTNFR1 and sTNFR2 Biomarker Profiling.
Implications for Maternal Cardiovascular Risk, Endothelial Dysfunction and Disease Monitoring.

EXECUTIVE SUMMARY

Twin-Twin Transfusion Syndrome (TTTS) is a severe placental vascular disorder affecting approximately 10-15% of monochorionic twin pregnancies and remains a significant cause of fetal and maternal morbidity. While clinical management predominantly focuses on fetal outcomes, increasing evidence suggests that TTTS is associated with a substantial maternal physiological response characterized by cardiovascular stress, systemic inflammation and endothelial dysfunction.

In this study, maternal serum concentrations of Heart-type Fatty Acid Binding Protein (H-FABP), Midkine, soluble Tumour Necrosis Factor Receptor-1 (sTNFR1) and soluble Tumour Necrosis Factor Receptor-2 (sTNFR2) were significantly altered in pregnancies complicated by TTTS compared with uncomplicated monochorionic twin pregnancies.

The biomarker profile observed demonstrates considerable overlap with pathways implicated in pre-eclampsia and other vascular pregnancy disorders, suggesting that maternal cardiovascular and inflammatory activation are important components of the disease process.

CLINICAL BACKGROUND

TTTS occurs as a consequence of unbalanced placental vascular anastomoses, resulting in chronic inter-twin haemdynamic imbalance. The associated placental dysfunction leads to profound fetal cardiovascular adaptations, but the maternal response has historically received less attention.

Women affected by TTTS have an increased risk of hypertensive disorders of pregnancy, particularly pre-eclampsia, suggesting common underlying mechanisms involving:

- ✔

ENDOTHELIAL DYSFUNCTION
- ✔

CARDIOVASCULAR STRESS
- ✔

SYSTEMIC INFLAMMATION
- ✔

ANGIOGENIC IMBALANCE

Biomarkers capable of reflecting these biological processes may improve our understanding of disease pathophysiology and potentially support maternal risk stratification in future clinical studies.

STUDY OVERVIEW

Maternal serum samples were obtained from women with:

- ✔

TWIN-TWIN TRANSFUSION SYNDROME (N = 51)
- ✔

UNCOMPLICATED MONOCHORIONIC DIAMNIOTIC TWIN PREGNANCIES (N = 29)

Samples were analysed before fetoscopic laser ablation (FLA), the standard treatment for TTTS, with a subset undergoing repeat sampling six hours after intervention.

The research used the Randox Laboratories RanChip Kidney Dysfunction array (EV4560), based on Biochip Array Technology, to analyse H-FABP, midkine, sTNFR1 and sTNFR2. The RanChip is a multiplex chemiluminescent immunoassay that enables simultaneous detection of multiple analytes on a single solid-phase biochip. RanChips were run on the Randox Evidence MultiSTAT (EV4115) and samples were analysed in duplicate by Randox Clinical Laboratory Services.

H-FABP: EVIDENCE OF MATERNAL CARDIOVASCULAR STRESS

KEY FINDING

Maternal H-FABP concentrations were substantially elevated in TTTS pregnancies:

Following fetoscopic laser ablation:

GROUP	H-FABP (ng/mL)	TIMEPOINT	H-FABP (ng/mL)
Controls	2.0	Pre-FLA	3.4
TTTS	3.4	Post-FLA	4.7

CLINICAL INTERPRETATION

H-FABP is a low molecular weight cytoplasmic protein released following myocardial injury and is recognised as a sensitive marker of cardiovascular stress.

Although concentrations observed in this cohort are not indicative of acute myocardial infarction, the significant elevation suggests increased maternal cardiovascular strain associated with TTTS. The further increase following laser therapy likely reflects an acute physiological response to intervention and altered haemodynamic conditions.

These findings support the concept that TTTS should be considered not only a fetal disease but also a maternal cardiovascular stress state.

MIDKINE: A MARKER OF ENDOTHELIAL ACTIVATION AND TISSUE RESPONSE

KEY FINDING

Midkine concentrations were significantly increased in TTTS:

Following intervention:

GROUP	Midkine (pg/mL)	TIMEPOINT	Midkine (pg/mL)
Controls	2504	Pre-FLA	3279
TTTS	3279	Post-FLA	4055

CLINICAL INTERPRETATION

Midkine is a heparin-binding growth factor involved in:

- ✔

ENDOTHELIAL ACTIVATION
- ✔

INFLAMMATORY SIGNALLING
- ✔

ANGIOGENESIS
- ✔

TISSUE REPAIR

Elevated concentrations in TTTS suggest activation of vascular stress pathways and adaptive responses to placental dysfunction. The marked post-procedural increase is consistent with an acute injury-response mechanism and may reflect endothelial remodelling following laser treatment.

Given Midkine’s emerging role in cardiovascular and inflammatory diseases, these findings support further investigation into its utility as a biomarker of maternal vascular dysfunction.

TNF PATHWAY ACTIVATION IN TTTS

sTNFR1

GROUP	sTNFR1 (ng/mL)
Controls	1.1
TTTS	1.2

sTNFR2

GROUP	sTNFR2 (ng/mL)
Controls	0.2
TTTS	0.4

TWO-FOLD ELEVATION IN TTTS

TIMEPOINT	sTNFR1 (ng/mL)
Pre-FLA	1.2
Post-FLA	1.3

CLINICAL INTERPRETATION

The TNF signalling pathway is a central mediator of inflammatory and endothelial responses. Soluble TNF receptors are widely recognised biomarkers of:

✔ CHRONIC INFLAMMATION

✔ CARDIOVASCULAR DISEASE

✔ PRE-ECLAMPSIA

✔ ENDOTHELIAL DYSFUNCTION

✔ RENAL DYSFUNCTION

The modest rise in sTNFR1, together with the pronounced two-fold elevation in sTNFR2, indicates substantial activation of inflammatory pathways in women with TTTS. Importantly, elevations of circulating soluble TNF receptors have been consistently associated with vascular dysfunction and adverse pregnancy outcomes in pre-eclampsia studies, suggesting parallel biological mechanisms.

INTERRELATIONSHIP OF CARDIOVASCULAR AND INFLAMMATORY PATHWAYS

A major strength of multiplex biomarker analysis is the ability to identify interactions between biological systems.

BIOMARKERS	Correlation Coefficient (ρ)
H-FABP vs sTNFR1	0.634
H-FABP vs sTNFR2	0.442

BIOMARKERS	Correlation Coefficient (ρ)
H-FABP vs Midkine	0.436
sTNFR1 vs sTNFR2	0.575

CLINICAL INTERPRETATION

The strong association between H-FABP and both soluble TNF receptors suggests a close relationship between:

✔ CARDIOVASCULAR STRESS

✔ SYSTEMIC INFLAMMATION

✔ ENDOTHELIAL ACTIVATION

These findings support a model in which placental dysfunction triggers a coordinated maternal response involving multiple overlapping biological pathways.

Rather than representing isolated biomarker abnormalities, the observed changes appear to reflect a unified disease process.

RELATIONSHIP TO PRE-ECLAMPSIA

Several findings in TTTS mirror observations previously reported in pre-eclampsia:

SHARED PATHOPHYSIOLOGICAL FEATURES

- ✔ ENDOTHELIAL DYSFUNCTION

✔ CARDIOVASCULAR STRESS
- ✔ ANGIOGENIC IMBALANCE

✔ ELEVATED TNF SIGNALLING
- ✔ INCREASED INFLAMMATORY ACTIVATION

This overlap may explain the increased incidence of maternal hypertensive disorders observed in TTTS pregnancies and reinforces the concept of a shared vascular pathophysiology.

From a clinical perspective, TTTS may provide a unique model through which maternal cardiovascular adaptation to placental disease can be studied.

CLINICAL INTERPRETATION

The results provide several important insights for clinicians and researchers:

1. MATERNAL DISEASE IS AN IMPORTANT COMPONENT OF TTTS

The biomarker profile demonstrates measurable maternal physiological disturbance beyond the fetal manifestations of disease.
2. CARDIOVASCULAR STRESS IS PRESENT BEFORE CLINICAL DETERIORATION

Elevated H-FABP suggests cardiovascular involvement may occur before overt maternal complications develop.
3. INFLAMMATORY PATHWAYS ARE ACTIVELY ENGAGED

The substantial increase in sTNFR2 supports a significant inflammatory contribution to disease pathogenesis.
4. DYNAMIC RESPONSES CAN BE MEASURED FOLLOWING INTERVENTION

Changes occurring within hours of laser therapy indicate that these biomarkers may have value for monitoring acute biological responses.
5. MULTIPLEX BIOMARKER PANELS PROVIDE GREATER BIOLOGICAL INSIGHT

Simultaneous assessment of cardiovascular, inflammatory and endothelial pathways offers a more comprehensive understanding of disease mechanisms than individual biomarkers alone.

WHAT THE RANCHIP APPROACH DEMONSTRATES IN TRANSLATIONAL RESEARCH

- ✔ Multiplex measurement can consolidate several biologically related markers into a single panel and sample workflow.
- ✔ A panel-based approach supports investigation of interconnected pathways rather than relying on a single biomarker in isolation.
- ✔ The Kidney Dysfunction RanChip enabled simultaneous measurement of four markers that contributed directly to the study’s cardiovascular and inflammatory pathway analysis.

THE PLATFORM – EVIDENCE MULTISTAT

The Evidence MultiSTAT provides a compact platform for the simultaneous detection of multiple biomarkers, delivering accurate, quantitative results in less than 60 minutes.

Minimal sample preparation and an integrated MultiSTAT cartridge, containing the reagents required for the chemiluminescent reaction, simplify the workflow from sample entry to result into just two steps. With no additional components required, the system supports straightforward operation while minimising the potential for human error.



CONCLUSIONS

Maternal circulating concentrations of H-FABP, Midkine, sTNFR1 and sTNFR2 are significantly altered in pregnancies complicated by Twin-Twin Transfusion Syndrome.

The observed biomarker profile demonstrates coordinated activation of cardiovascular, inflammatory and endothelial pathways, supporting the concept that TTTS is associated with a systemic maternal disease response in addition to fetal pathology.

The strong associations between markers of cardiovascular stress and inflammatory activation suggest a biologically integrated pathway that shares important features with pre-eclampsia.

These findings provide new insights into maternal adaptation to placental vascular disease and highlight the value of multiplex biomarker profiling in advancing our understanding of obstetric disorders.

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