Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

Diploma Thesis

MEDIZINISCHE UNIVERSITÄT WIEN

Changes in the biological function of peripheral mononuclear cells in diabetes mellitus

Elisabeth Simader

Betreuer
Univ.-Prof. Dr. med. univ. Hendrik Jan Ankersmit, MBA
Ao.Univ.Prof. Dr. med univ. Alois Geßl
Dr. med. univ. Matthias Zimmermann

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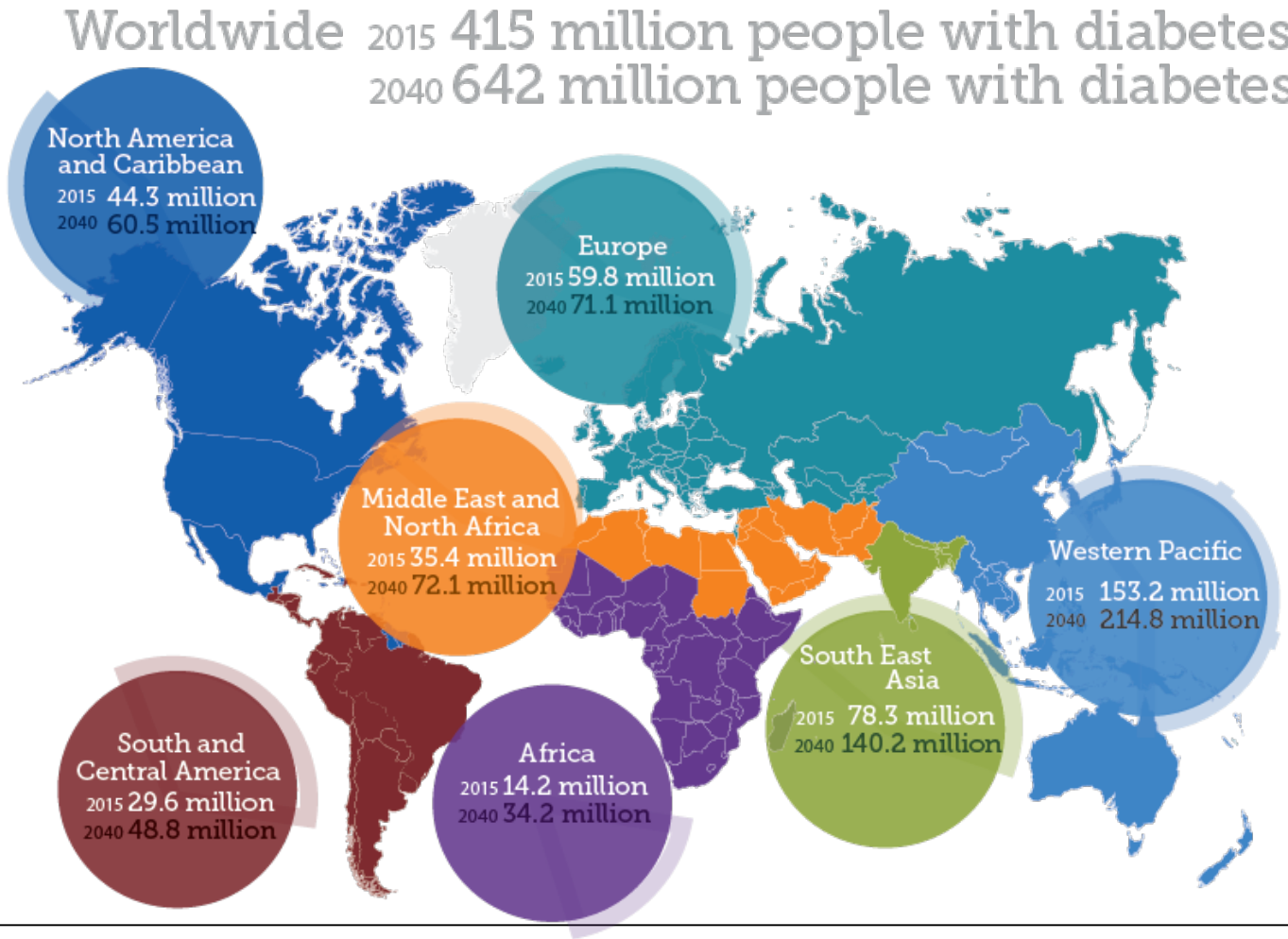
Background Diabetes



International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org

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Diagnosis: Diabetes mellitus

Fasting plasma glucose (FPG)

≥126mg/dL (7.0 mmol/l)

2h-plasma-glucose (OGTT)

≥200mg/dL (11.1mmol/l)

HbA1C ≥6.5%

Random plasma glucose

≥200mg/dL (11.1mmol/l)

American Diabetes Association. *Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers.* Clin Diabetes, 2016. **34**(1): p. 3-21.

American Diabetes Association. *Standards of medical care in diabetes--2014.* Diabetes Care, 2014. **37 Suppl 1**: p. S14-80.

Background

Diagnosis: Impaired fasting glucose

Fasting plasma glucose (FPG)

100 mg/dL (5.6 mmol/L) to
125 mg/dL (6.9 mmol/L)

2h-plasma-glucose (OGTT)

140 mg/dL (7.8 mmol/L) to
199 mg/dL (11.0 mmol/L)

HbA1C ≥5.7%

American Diabetes Association. *Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers.* Clin Diabetes, 2016. **34**(1): p. 3-21.
American Diabetes Association. *Standards of medical care in diabetes--2014.* Diabetes Care, 2014. **37 Suppl 1**: p. S14-80.

Classification

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

A. Immune mediated

B. Idiopathic

American Diabetes Association. *Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers.* Clin Diabetes, 2016. **34**(1): p. 3-21.
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II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

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I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
   A. Immune mediated
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II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types

A. Genetic defects of β-cell function
B. Genetic defects in insulin action
C. Diseases of the exocrine pancreas
D. Endocrinopathies
E. Drug or chemical induced
F. Infections
G. Uncommon forms of immune-mediated diabetes
H. Other genetic syndromes sometimes associated with diabetes

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American Diabetes Association. *Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers.* Clin Diabetes, 2016. **34**(1): p. 3-21.
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   D. Endocrinopathies
   E. Drug or chemical induced
   F. Infections
   G. Uncommon forms of immune-mediated diabetes
   H. Other genetic syndromes sometimes associated with diabetes

IV. Gestational diabetes mellitus

American Diabetes Association. *Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers.* Clin Diabetes, 2016. **34**(1): p. 3-21.
American Diabetes Association. *Standards of medical care in diabetes--2014.* Diabetes Care, 2014. **37 Suppl 1**: p. S14-80.

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Complications

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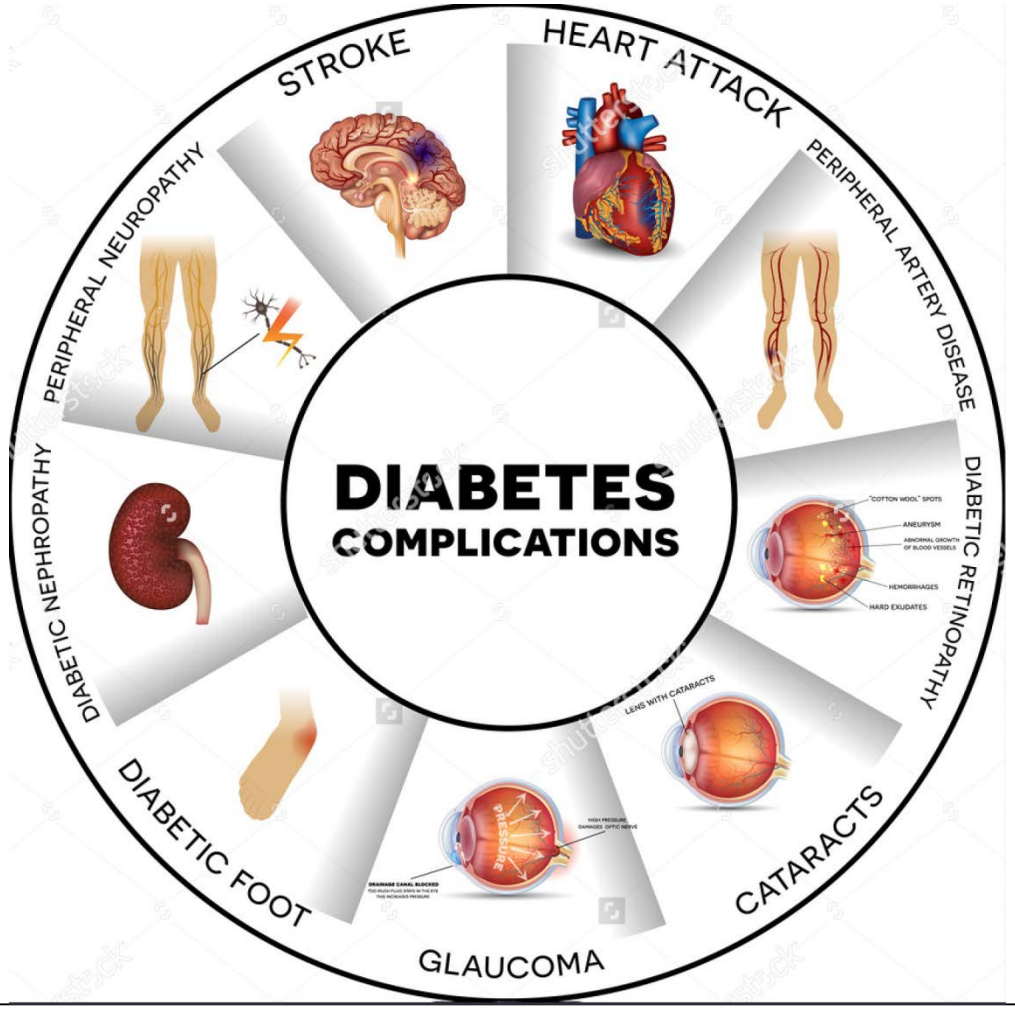

http://www.shutterstock.com/pic-341076281/stock-vector-diabetes-complications-affected-organs-diabetes-affects-nerves-kidneys-eyes-vessels-heart.html

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Complications

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Microvascular complications

- Diabetic kidney disease
- Diabetic retinopathy
- Neuropathy
- Leg ulcer/ peripheral arterial disease

Macrovascular complications

- Myocardial infarction
- Stroke
- Transient ischemic attack

American Diabetes Association. Standards of Medical Care in Diabetes-2016 Abridged for Primary Care Providers. Clin Diabetes, 2016. ***34****(1): p. 3-21.*
https://sites.google.com/a/guhsd.net/the-cardiovascular-system/

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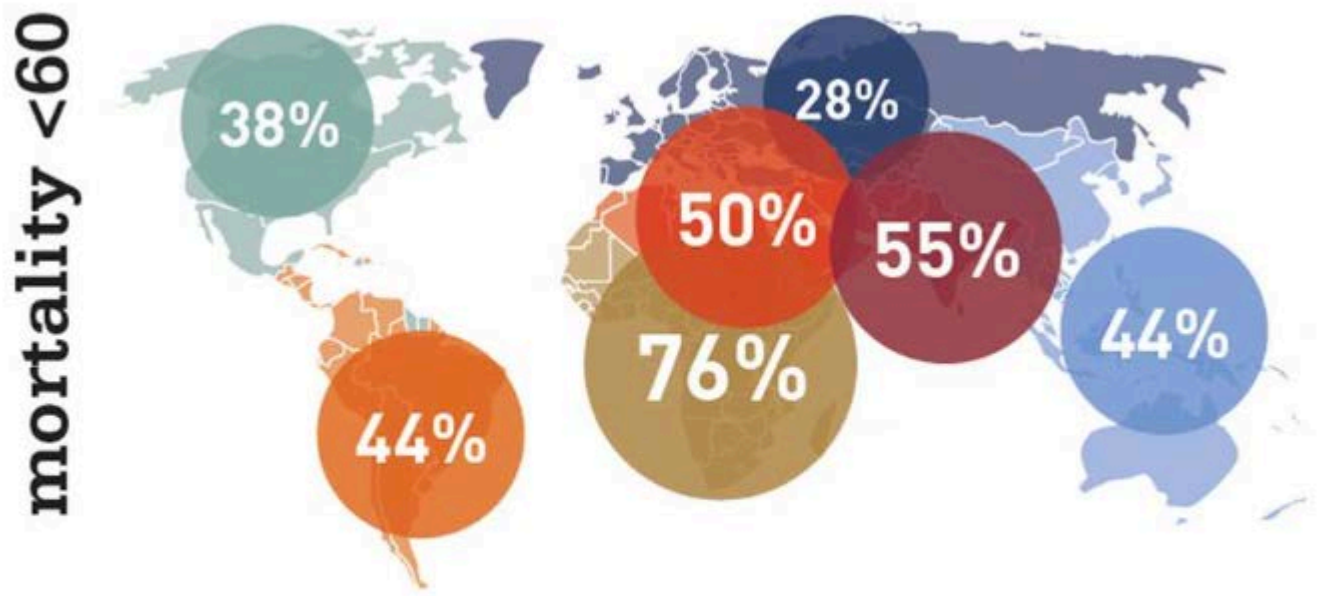
Complications of diabetes



Proportion of deaths due to diabetes in people under 60 years of age, 2013

IDF Releases New Dire Diabetes Stats and Projections*11/15/2013 02:37 pm ET* | ***Updated*** *Jan 23, 2014 ; http://www.huffingtonpost.com/riva-greenberg/diabetes-stats_b_4273505.html*

International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015. http://www.diabetesatlas.org

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Background

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Macrophage Migration Inhibitory Factor (MIF) is associated with complex coronary lesions. [1]

Gene silencing of MIF leads to attenuation of atherosclerotic lesions.[2]

Association of serum levels of matrix metalloproteinase-9 (MMP-9) with myocardial infarction. [3]

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1 Hao, Y., S.L. Yi, and J.Q. Zhong, Serum macrophage migration inhibitory factor levels are associated with angiographically complex coronary lesions in patients with coronary artery disease. Genet Test Mol Biomarkers, 2015. **19**(10): p. 556-60.

2 Sun, H., et al., Attenuation of atherosclerotic lesions in diabetic apolipoprotein E-deficient mice using gene silencing of macrophage migration inhibitory factor. J Cell Mol Med, 2015. ***19matrix metalloproteinase-9*** *(4): p. 836-49.*

3 Jefferis, B.J., et al., *Prospective study of matrix metalloproteinase-9 and risk of myocardial infarction and stroke in older men and women. Atherosclerosis, 2010.* ***208****(2): p. 557-63.*

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Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 4782–4787, April 1997
Physiology

Insulin secretion is regulated by the glucose-dependent production of islet β cell macrophage migration inhibitory factor

(diabetes/endocrine pancreas/gene regulation/cytokine/MIF)

GÉRARD WAEBER*†, THIERRY CALANDRA‡, RAPHAEL RODUIT§, JACQUES-ANTOINE HAEFLIGER*, CHRISTOPHE BONNY*, NANCY THOMPSON*, BERNARD THORENS§, EVELYNE TEMLER¶, ANDREAS MEINHARDT‖, MICHAEL BACHER‡, CHRISTINE N. METZ‡, PASCAL NICOD*, AND RICHARD BUCALA‡

*Department of Internal Medicine B and ¶Division of Endocrinology and Metabolism, University Hospital, CHUV-1011 Lausanne, Switzerland; §Pharmacology and Toxicology Institute, University of Lausanne, 1005 Lausanne, Switzerland; ‖Department of Anatomy and Cell Biology, Philipps University of Marburg, 35033 Marburg, Germany; and ‡The Picower Institute for Medical Research, Manhasset, New York 11030

Communicated by Helen M. Ranney, Alliance Pharmaceutical Corp., San Diego, CA, March 3, 1997 (received for review December 9, 1996)

ABSTRACT **Macrophage migration inhibitory factor (MIF), originally identified as a cytokine secreted by T lymphocytes, was found recently to be both a pituitary hormone and a mediator released by immune cells in response to glucocorticoid stimulation. We report here that the insulin-secreting β cell of the islets of Langerhans expresses MIF and that its production is regulated by glucose in a time- and concentration-dependent manner. MIF and insulin colocalize by immunocytochemistry within the secretory granules of the pancreatic islet β cells, and once released, MIF appears to regulate insulin release in an autocrine fashion. In perifusion studies performed with isolated rat islets, immunoneutralization of MIF could function in other contexts as a protein mediator within the endocrine system. We performed immunohistochemical studies in rat tissues to examine the localization of MIF within endocrine tissues. In this report, we show that abundant quantities of MIF mRNA and protein are detected in primary rat islets of Langerhans. MIF is highly expressed in several insulin-secreting cell lines, colocalizes with insulin-containing secretory granules, and is secreted in response to glucose stimulation in a time- and concentration-dependent manner. Immunoneutralization of MIF in perifusion studies or the constitutive expression of MIF antisense RNA in an insulin-secreting cell line reduced significantly the first and**

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Diabetologia (2008) 51:276–284
DOI 10.1007/s00125-007-0800-3

ARTICLE

Effect of macrophage migration inhibitory factor (MIF) gene variants and MIF serum concentrations on the risk of type 2 diabetes: results from the MONICA/KORA Augsburg Case–Cohort Study, 1984–2002

C. Herder · N. Klopp · J. Baumert · M. Müller · N. Khuseyinova · C. Meisinger · S. Martin · T. Illig · W. Koenig · B. Thorand

Received: 6 June 2007 / Accepted: 19 July 2007 / Published online: 22 August 2007

Abstract
Aims/hypothesis Macrophage migration inhibitory factor (MIF) is a central mediator of innate immunity. Our aim was to investigate the triangular association between *MIF* women), we determined MIF serum levels at baseline and genotyped four *MIF* single nucleotide polymorphisms (SNPs).
Results The C allele of SNP rs1007888 (3.8 kb 3′ of the

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Aims of the study

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(a) the analysis of changes in the physiological function, gene expression and secretion pattern of PBMCs in patients with diabetes

(b) the identification of possible correlations with disease severity.

Study population

Group 1: Newly diagnosed diabetes, without medication (n=11)

Group 2: Diabetes mellitus, under therapy (n=15)

Group 3: Impaired fasting glucose (prediabetes) (n=13)

Group 4: Healthy controls (n=15)

Exclusion criteria:

Chronic inflammatory diseases, acute infections, malign neoplasia in the last 5 years, leukocytopenia, leukocytosis, pregnancy, chronic heart insufficiency, peripheral arterial disease, unstable angina pectoris

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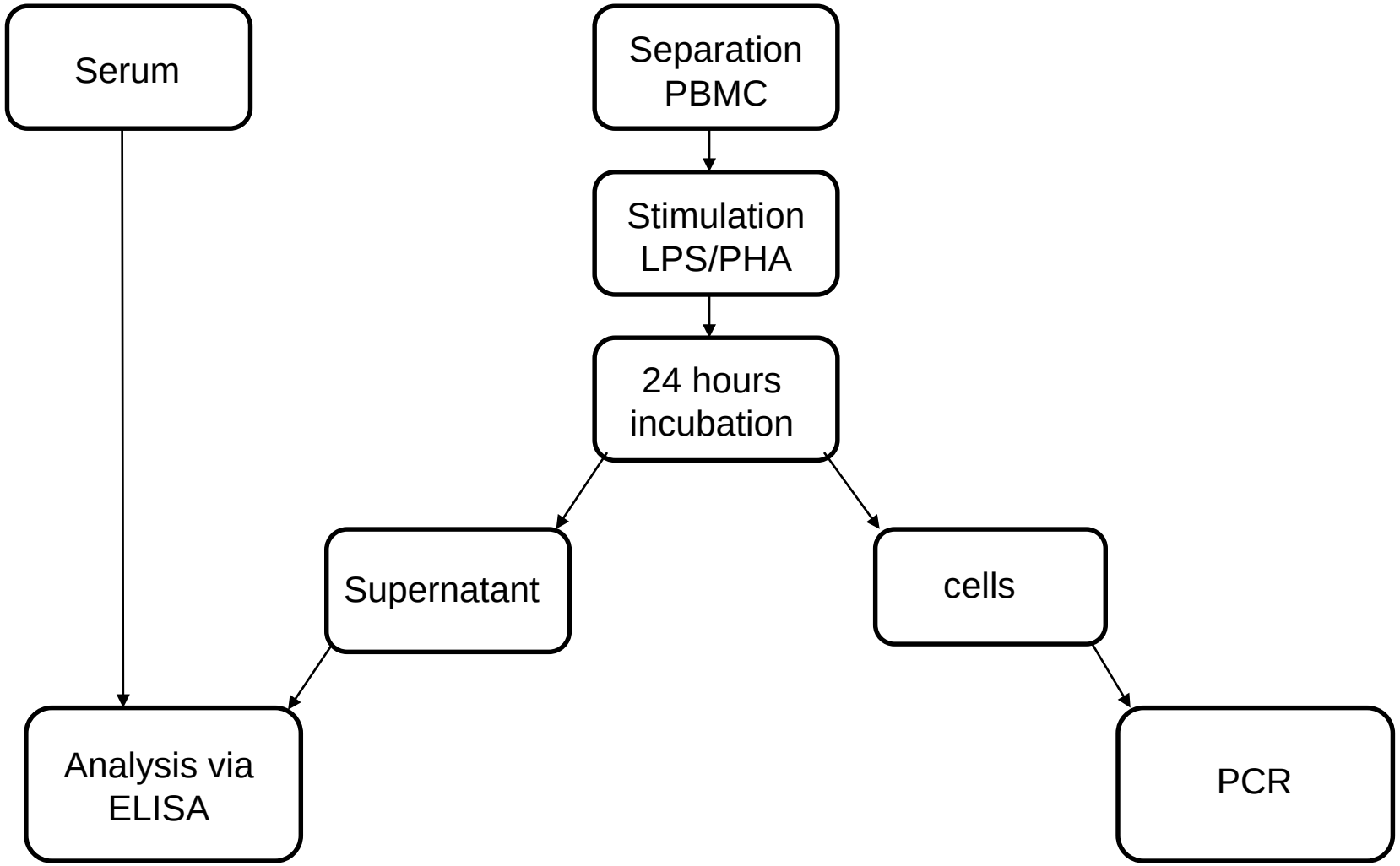
Study Design



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PBMC-Separation

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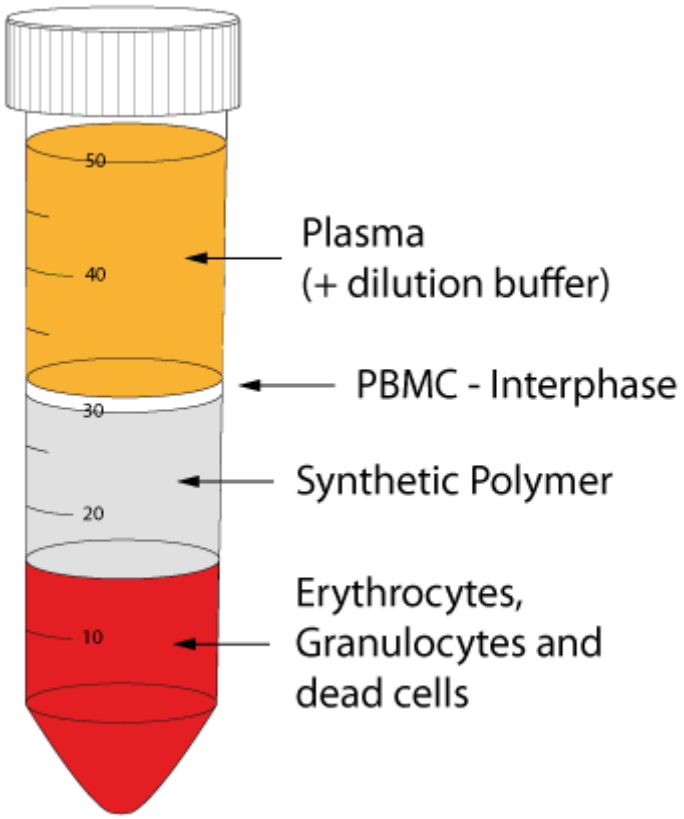

- Separation with Ficoll-Paque
- Buffy coat washed 2x times
- Incubated for 24h

Adapted from: pluriselect
https://www.pluriselect.com/pbmc.html

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Methods

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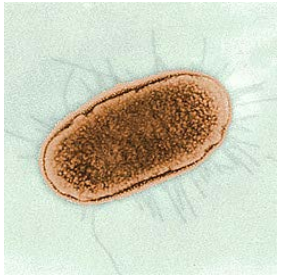
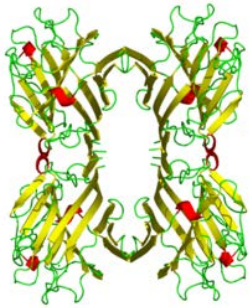
Endotoxin
Lipopolysaccharide



Stimulation with LPS →

Production of Inflammatory Cytokines
IL-1α, IL-1β, IL-6, TNFα, **MIF**

Phytohaemagglutinin



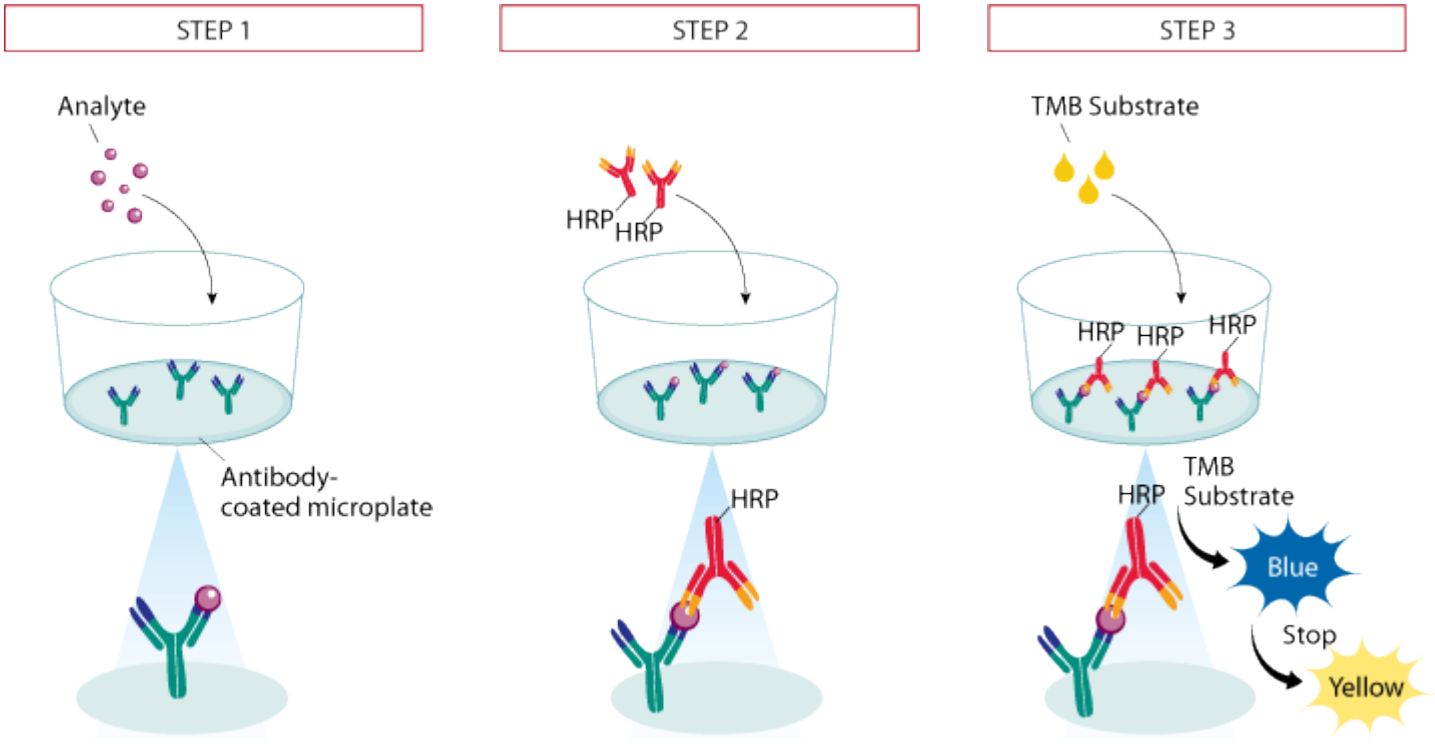
Stimulation with PHA →

Induction of MMP-9

Mildner M, Storka A, Lichtenauer M, et al. Primary sources and immunological prerequisites for sST2 secretion in humans. *Cardiovasc Res* 2010; 87(4):769-77.

ELISA

Enzyme-linked immunosorbent assays



Adapted from: DuoSet® ELISA Development Systems;
http://www.woongbee.com/0NewHome/RnD/ELISA_HA/Duoset.htm

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Results

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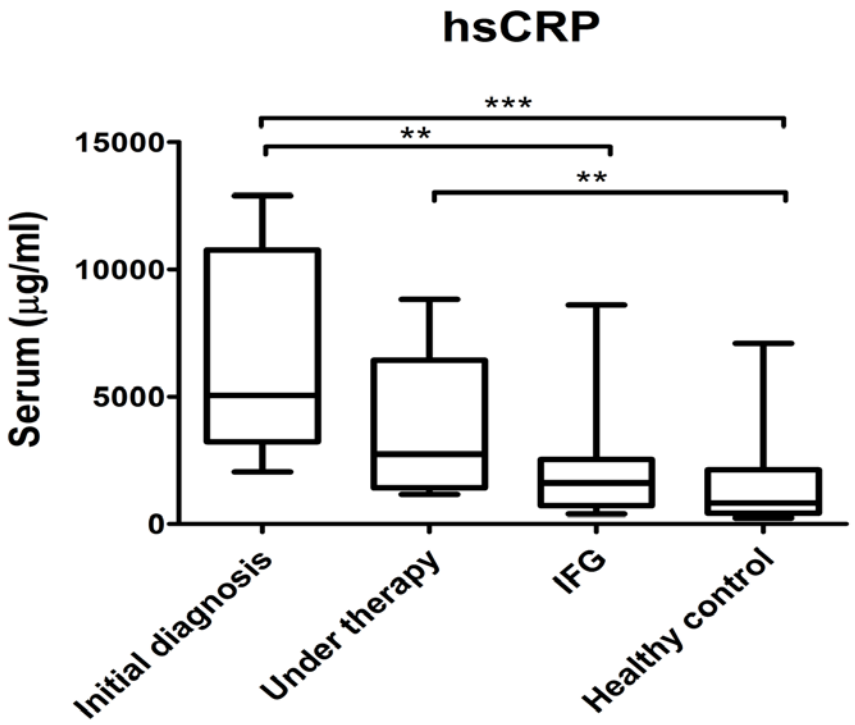

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Results

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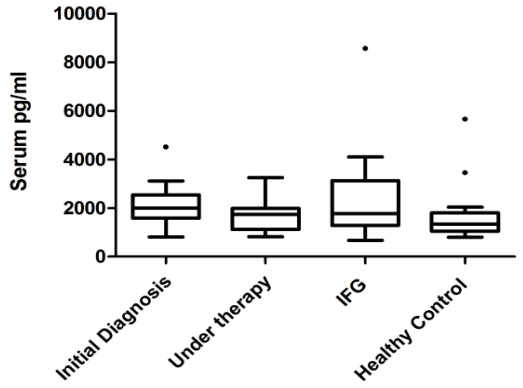
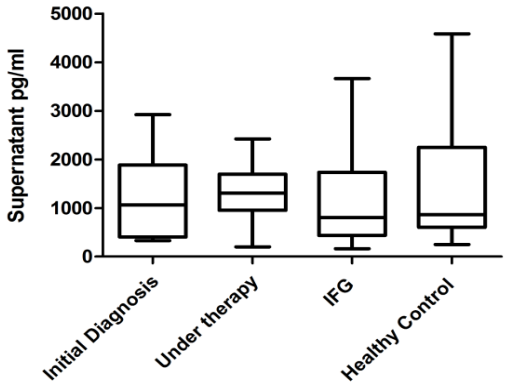
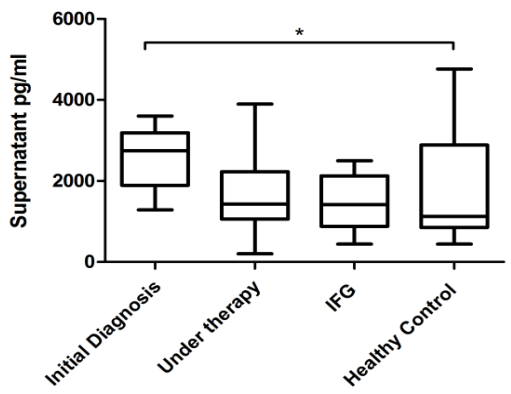
a)



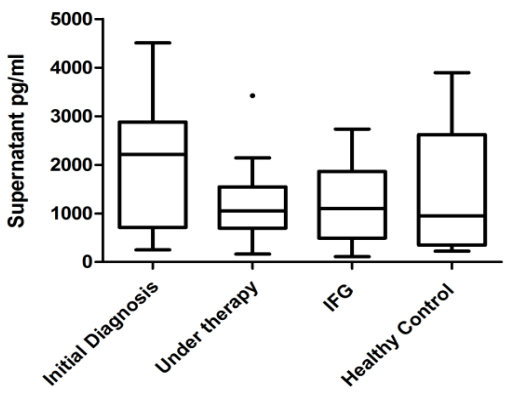
b)



c)



d)



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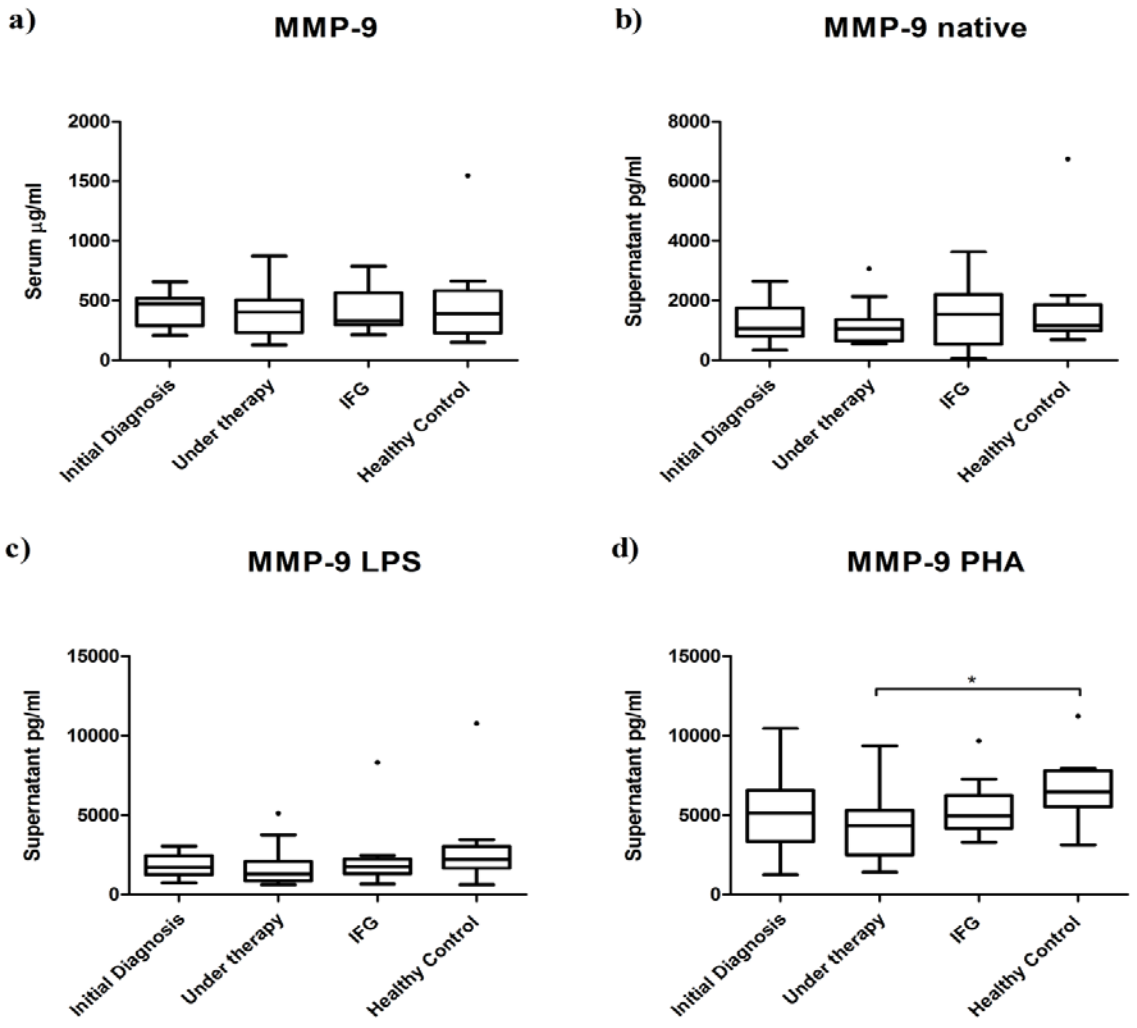

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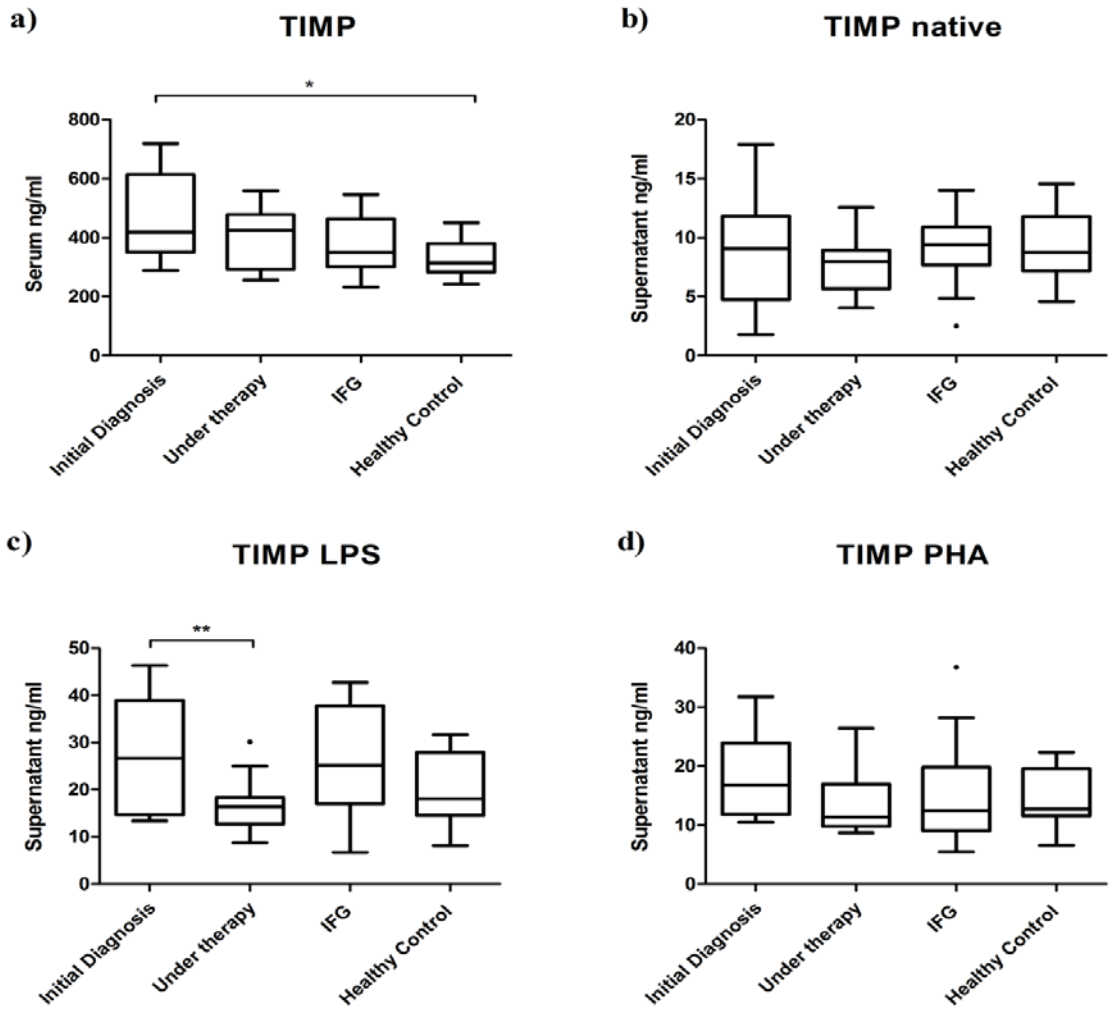

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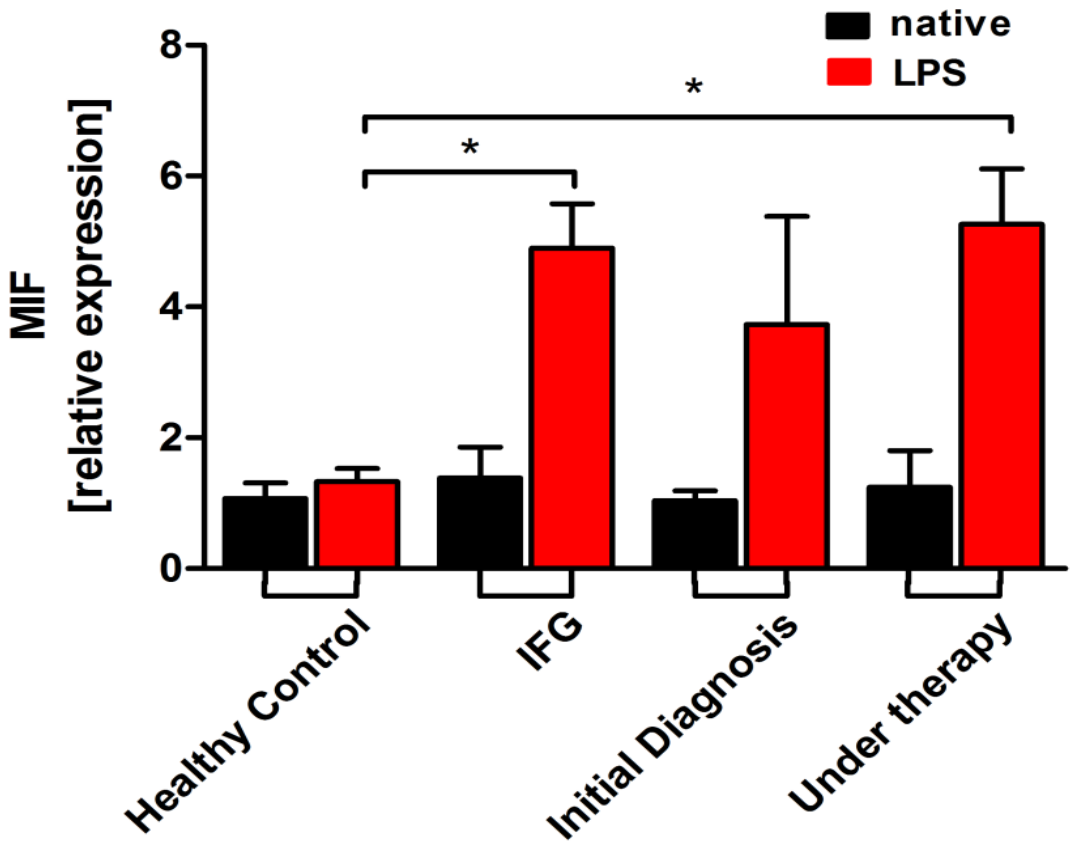

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Conclusion

Diabetic patients have higher levels of hsCRP, indicating a higher cardiovascular risk.

In response to endotoxins PBMCs of newly diagnosed diabetic patients secrete higher levels of MIF.

Gene expression of MIF is elevated in prediabetic and diabetic patients, compared to healthy controls.

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Special Thanks

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Medical University of Vienna

Christian Doppler Laboratory
for Cardiac and Thoracic Diagnosis and Regeneration

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Alois Geßl

Department of Dermatology

Michael Mildner
Tanja Berger

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Thank you for your attention