Immunometric and Functional Measurement of Endogenous Vasoinhibin in Human Sera

Magdalena Zamora[1,2]*, David Harris[1]*, Nils Davies[1], Johannes Ebnet[1], Peter Radermacher[3], Cosima Brucker[4], Christiane Waller[5], Juan Pablo Robles[2], Thomas Bertsch[1]†, Carmen Clapp[2]†, Jakob Triebel[1]†

1| Institute for Clinical Chemistry, Laboratory Medicine and Transfusion Medicine, Nuremberg General Hospital & Paracelsus Medical University, Nuremberg, Germany **2|** Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México **3|** Institute of Anesthesiological Pathophysiology and Process Engineering, University of Ulm, Ulm, Germany **4|** Department of Gynecology and Obstetrics, Nuremberg General Hospital & Paracelsus Medical University, Nuremberg, Germany **5|** Department of Psychosomatic Medicine and Psychotherapy, Nuremberg General Hospital & Paracelsus Medical University, Nuremberg, Germany; *These authors contributed equally to this work and share first authorship †These authors share senior authorship

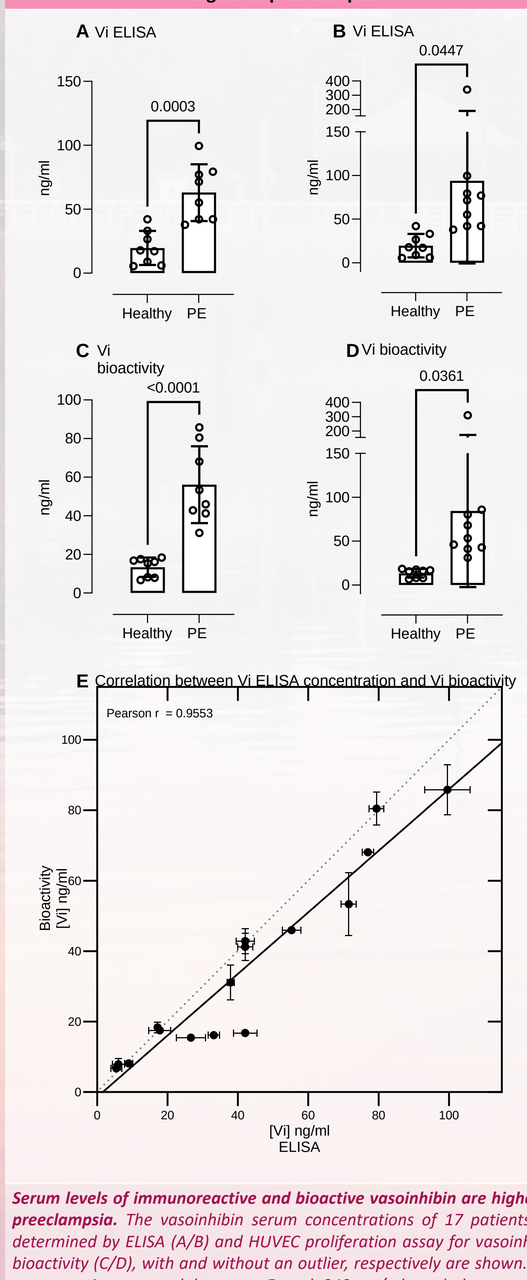
INTRODUCTION

Circulating levels of the antiangiogenic protein vasoinhibin, a fragment of prolactin, are of interest in vasoproliferative retinopathies, preeclampsia, and peripartum cardiomyopathy, but are unknown due to the lack of a quantitative assay. Here, human serum samples were investigated for the concentration and bioactivity of vasoinhibin using a novel enzyme-linked immunosorbent assay (ELISA) for human vasoinhibin employing an anti-vasoinhibin monoclonal antibody, a human umbilical vein endothelial cells (HUVEC) proliferation assay and a chick chorioallantoic membrane (CAM) angiogenesis assay.

RESULTS

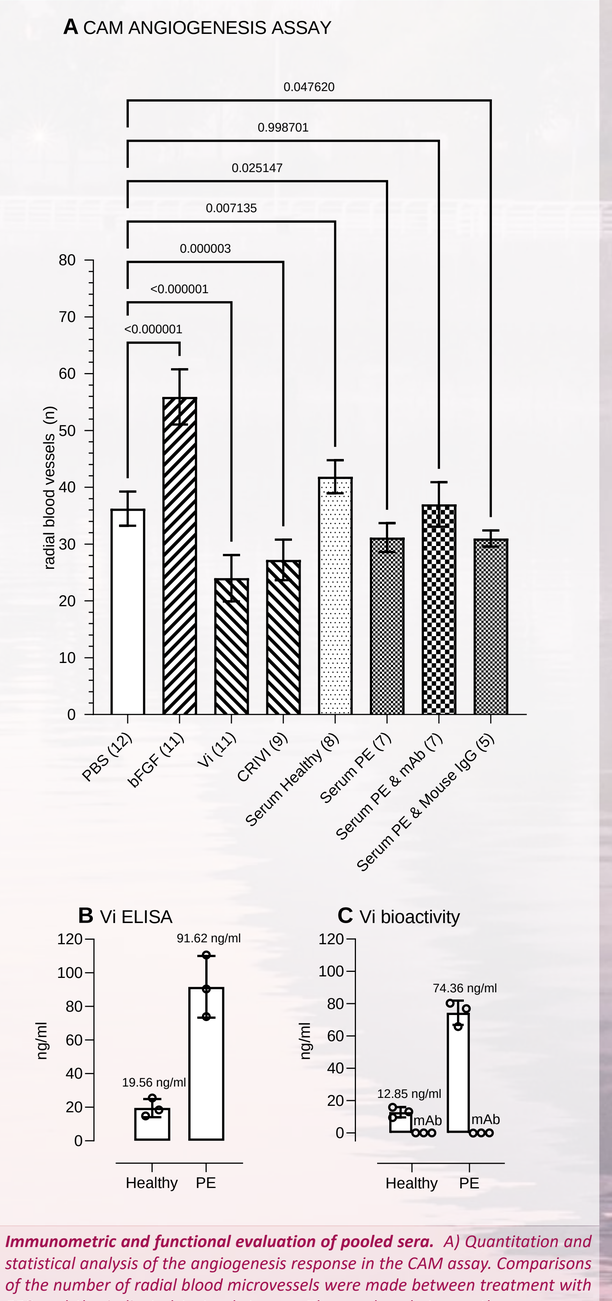
Serum samples from 17 pregnant women with and without preeclampsia and pregnancy induced hypertension demonstrated endogenous vasoinhibin concentrations in the range between 5 and 340 ng/ml. Vasoinhibin levels were significantly higher in preeclampsia serum compared to healthy pregnancy serum (mean 63.09 vs. 19.67 ng/ml, p=0.0003), as was the bioactivity of vasoinhibin determined by HUVEC proliferation (56.12 vs. 13.38 ng/ml, p=<0.0001). There was a correlation between the concentrations of vasoinhibin measured by ELISA and by HUVEC proliferation (Pearson r=0.95, p=<0.0001). Healthy serum demonstrated a proangiogenic effect in the CAM assay (p=<0.05, compared to a PBS control), while serum from preeclamptic patients demonstrated an antiangiogenic action (p=<0.05 vs. PBS control), as did recombinant human vasoinhibin and a synthetic circular retro-inverse vasoinhibin analog (CRIVi45-51). The antiangiogenic effects in the CAM assay and the inhibition of HUVEC proliferation were abolished by the addition of the ELISA anti-vasoinhibin monoclonal antibody but not by mouse IgG.

Serum levels of immunoreactive and bioactive vasoinhibin are higher in preeclampsia



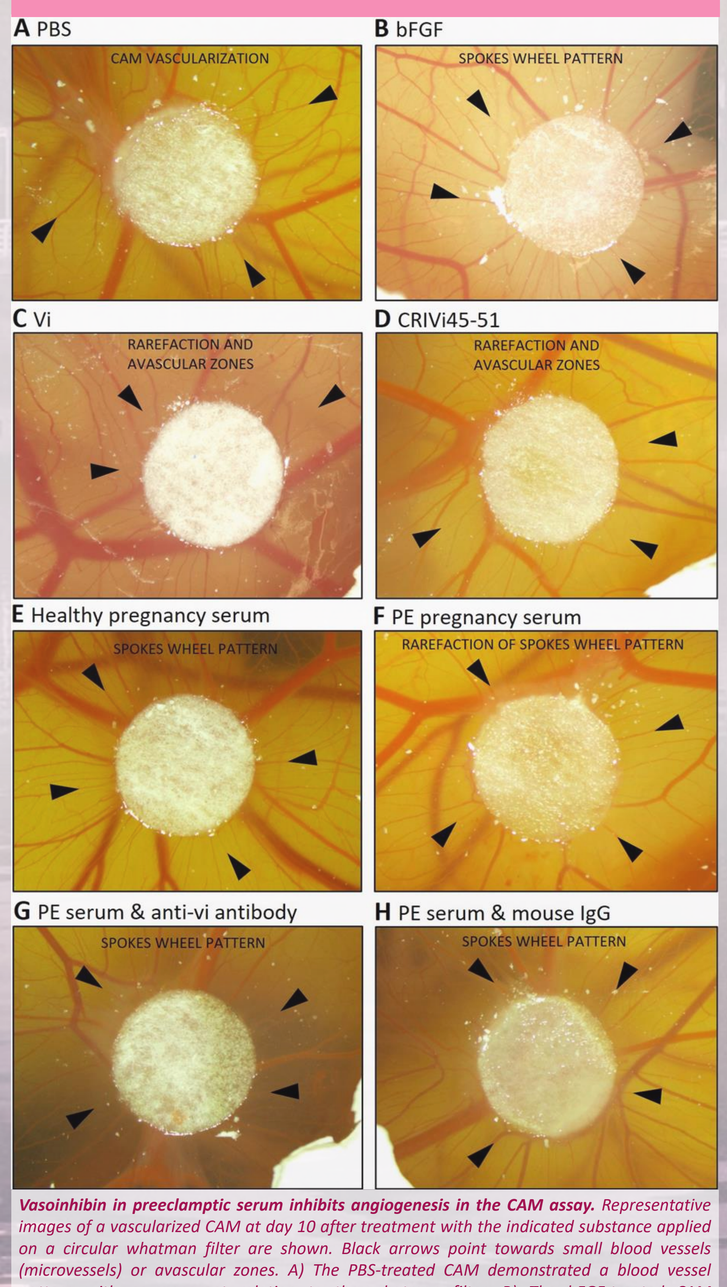
Serum levels of immunoreactive and bioactive vasoinhibin are higher in preeclampsia. *The vasoinhibin serum concentrations of 17 patients, as determined by ELISA (A/B) and HUVEC proliferation assay for vasoinhibin bioactivity (C/D), with and without an outlier, respectively are shown. The concentrations ranged between 5 and 340 ng/ml, and demonstrated significant differences between the healthy and the preeclamptic (PE) group as indicated (Student's t-test), values are means ± SD, n ≥ 8. E) There was a correlation between the vasoinhibin concentrations measured by the ELISA and the vasoinhibin concentrations determined in the HUVEC proliferation assay (Pearson r=0.95, p=<0.0001, excluding outlier).*

Immunometric and functional evaluation of pooled sera



Immunometric and functional evaluation of pooled sera. *A) Quantitation and statistical analysis of the angiogenesis response in the CAM assay. Comparisons of the number of radial blood microvessels were made between treatment with PBS and the indicated test substances. The p-values between the groups are indicated over each bracket. The number of CAMs in each group is indicated in brackets after the group designation. PBS = phosphate buffered saline, bFGF = basic fibroblast growth factor, Vi = vasoinhibin, CRIVi45-51 = cyclic retroinverse vasoinhibin, PE = preeclampsia pooled serum, IgG = mouse immunoglobulin G. B) Measurement of Vi levels by ELISA and by HUVEC proliferation assay (C) of the healthy and PE pooled sera. The vasoinhibin activity in both serum pools was <LOD when the serum was co-incubated with the anti-vasoinhibin antibody (mAb). Values are means ± SD.*

Vasoinhibin in preeclamptic serum inhibits angiogenesis in the CAM assay



Vasoinhibin in preeclamptic serum inhibits angiogenesis in the CAM assay. *Representative images of a vascularized CAM at day 10 after treatment with the indicated substance applied on a circular whatman filter are shown. Black arrows point towards small blood vessels (microvessels) or avascular zones. A) The PBS-treated CAM demonstrated a blood vessel pattern with no apparent relation to the whatman filter. B) The bFGF-treated CAM demonstrated a spokes wheel like pattern, in which microvessels radially converge towards the whatman filter. C) A rarefication of radial microvessels with larger avascular zones was observed when the CAM was treated with recombinant vasoinhibin or D) CRIVi45-51, a vasoinhibin circular analog. E) Serum from healthy pregnant women induced a spokes wheel like pattern with an increase in radial microvessels. F) Radial microvessels in the CAM treated with serum from patients with preeclampsia are at PBS level, and G) increase after treatment with anti-vasoinhibin monoclonal antibody. H) Serum from patients with preeclampsia enriched with mouse IgG is comparable to preeclampsia serum alone. PBS = phosphate buffered saline, bFGF = basic fibroblast growth factor, Vi = vasoinhibin, CRIVi45-51 = cyclic retroinverse vasoinhibin, PE = preeclampsia, IgG = mouse immunoglobulin G.*

CONCLUSIONS

These results demonstrate the first quantitation of endogenous, bioactive vasoinhibin in human sera and its elevated levels and antiangiogenic activity in sera from women with preeclampsia. The development and implementation of a quantitative assay for vasoinhibin overcomes a long-standing barrier and opens the perspective to a thorough clinical verification of vasoinhibin as a relevant biomarker.

AUTHOR INFORMATION

Jakob Triebel, MD
ORCID iD 0000-0002-1989-8645
e-mail: jakob.triebel@gmx.de
X: jakob_triebel

BES2023 P--111
Reproductive Endocrinology
Jakob Triebel
Poster presented at: SfE BES GLASGOW 2023
PosterSessionOnline.com SPREADING KNOWLEDGE