Predictive molecular profiling in blood of healthy vasospastic individuals: clue to targeted prevention as personalised medicine to effective costs

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Abstract Paradigm change from late interventional approach to predictive diagnostics followed by targeted prevention before manifest pathology, presents innovative concept for advanced healthcare. Preselection of healthy but pathology-predisposed individuals is the primary task in the overall action. Vasospasm is a frequent syndrome defined as an inappropriate constriction or insufficient dilatation in microcirculation. Vasospastic individuals are considered as healthy subpopulation predisposed to several pathologies including neurodegeneration. Clinical observations, subcellular imaging and “gene hunting”-investigations provide evidence for vasospasm as predisposition to glaucoma; development of further related pathologies cannot be excluded. Predictive molecular-profiling in blood can specify individual predisposition for effective prevention.

Keywords Predisposition in healthy individuals · Vascular deregulation · Predictive diagnostics · Blood biomarkers · Targeted prevention · Personalised medicine

Vasospastic Syndrome: definition, prevalence, detection

Vasospastic Syndrome or vascular deregulation (VD) is defined as an inappropriate constriction or insufficient dilatation in the microcirculation (see Fig. 1).

Generally, vasospasm is considered as primary and secondary one. Secondary VD is due to other diseases such as autoimmune one. Primary VD is prevalent in younger subpopulation, can potentially predispose to several disorders being, therefore, particularly attractive for predictive diagnostics and individualised treatment. Primary VD demonstrates following particularities:

- it occurs more frequent in females manifested in puberty and moderating with age
- this phenomenon is even more frequent in Japanese population compared to Caucasian [2]
- usually academics are more affected by VD [3]
- to clinical signs belong an inborn increased sensitivity to any kind of stress provocation (mechanical, cold, emotion, etc.), altered drug sensitivity, frequently cold extremities, altered sleep behaviour, reduced feeling of thirst, low blood-pressure, reduced body-mass-index, more frequent migraine compared to general population [1, 4, 5]
- Compared to general population, vasospastic individuals tend to a meticulous personality and successful professional career [6].
A valuable diagnostic tool for the ascertainment of vasospastic diathesis is the nailfold capillary microscopy (see Fig. 2).

The best known blood-related risk factor is an increased plasma level of endothelin-1 [1]. Since Vasospastic Syndrome is a frequent phenomenon in young subpopulations, this makes the task of prediction and targeted prevention of “down-stream” related pathologies particularly attractive from several points of view including economical aspects.

**What is the impact of vascular deregulation in glaucoma pathology?**

A wealth of literature points to the importance of haemodynamics in glaucoma pathology. Vasospasm is frequently observed in glaucoma patients [1, 7]. Ocular ischemia resulting from blood-flow deficits may play a major role in the initiation of glaucoma: hypoxia, followed by high secretion of excitatory amino acids and elevated levels of intracellular calcium results in the process of retinal ganglion cell death [8–10]. In our previous studies, we have demonstrated stable alterations in gene expression of circulating leucocytes isolated from glaucoma patients compared to healthy controls [11–14]. Further, significant similarities in expression profiles of circulating leucocytes between vasospastic individuals and glaucoma patients have been recently published [15]. However, the same publication reports also significant dissimilarities of molecular patterns as compared to both glaucoma patients and healthy controls; consequently, a development of both degenerative and non-degenerative pathologies different from glaucomatous optic nerve degeneration but related to primary vasospasm cannot be excluded in vasospastic individuals.

**What is the potential impact of prediction and prevention of glaucoma in healthy vasospastic individuals?**

Worldwide, 67 million patients are affected by the neurodegenerative eye disease glaucoma. Glaucomatous optic neuropathy (GON) is the second leading cause of permanent vision loss. GON is a chronic degenerative process, the onset of which is not possible to monitor by currently existing diagnostic tools. Early treatment has been reported to be highly beneficial for well-timed treatment measures to slow-down the disease progression [16]. As review in this journal-issue [17], molecular pathomechanisms of glaucoma demonstrate both a considerable overlap and remarkable particularities to some other neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases. Thus versus controls, the neuronal thread protein (NTP) demonstrates enhanced expression levels in glaucoma, patients with Down Syndrome, Alzheimer’s and some other neurodegenerative diseases indicating the axonal lesions. However, whereas the accumulation of TAU-protein is characteristic for Alzheimer’s disease and other tauopathies, glaucoma patients do not demonstrate an increase in the target protein versus controls [16, 18]. Therefore, a potential predisposition of vasospastic individ-
uals to related pathologies should be thoroughly examined. In this context, molecular pathways involved in vasospastic deregulation should be investigated from viewpoint of

- identification of possible similarities as well as dissimilarities in molecular pathways between healthy vasospastic individuals and potential related pathologies developed later in life
- specificity for predictive diagnostics of glaucoma pathology in predisposed vasospastic individuals should be strictly validated against several control groups including other neurodegenerative diseases
- selection of molecular targets should be performed for vasospastic individuals in favour of non-invasive (blood test) diagnostic approaches followed by personalised treatment towards individual predisposition to single pathologies.

A monitoring of the pathology-specific molecular patterns is particularly valuable to develop reliable diagnostic approaches before the manifest pathology. Predictive tests can specify individual predisposition for well-timed preventive measures.

**Similarities in subcellular images of DNA-damage and -repair capacity in vasospasm and glaucoma**

Research work focused on the *ex vivo* comparative investigations of DNA damage in circulated leucocytes (CL) isolated from patients with glaucoma demonstrated significantly enhanced DNA damage compared to both healthy vasospastic and non-vasospastic individuals [19]. Comparative “Comet Assay” analysis revealed patterns of comets typical for glaucoma patients as shown in Fig. 3.

Although DNA damage in the vasospastic non-glaucomatous group is not found to be significantly increased versus healthy controls, DNA from vasospastic individuals showed highly group-specific comet-patterns with the degree of damage intermediate between healthy controls and glaucoma patients. These findings indicate “comet assay” profiling of DNA-damage in CL as a potentially powerful tool for the non-invasive early/predictive molecular diagnostics of glaucoma disease in vasospastic individuals [18]. Furthermore, un repaired DNA-damage in vasospastic individuals can lead to several pathologies different from glaucomatous optic nerve degeneration. This predisposition should be thoroughly investigated and the specificity of “Comet Assay”-patterns of vasospastic individuals should be validated comparing with patterns of other degenerative and non-degenerative pathologies. Thus, “Comet Assay”-analysis as a suitable tool for biomarkers has also been suggested for another neurodegenerative disorder—Alzheimer’s disease [21]. “Comet Assay”-analysis reveals enhanced DNA damage in both high- and normal-tension glaucoma [19]. Whether the level of DNA-damage correlates with disease severity, or not remains currently unclear. Further studies should also evaluate, whether a significant increase in DNA damage of leucocytes of glaucoma patients is caused by either disease specific stress factors, such as local ischemic/reperfusion events, and/or decreased capacity of DNA-repair machinery. There is some evidence for both eventualities: simultaneous up-regulation of *p53* (stress regulated gene) and down-regulation of *XPGC* (essential member of DNA-repair machinery) have been *ex vivo* demonstrated in CL of glaucoma patients [22] and represent potential molecular blood markers for the disease.

**Similarities in expression patterns detected in circulating leucocytes of vasospastic individuals and glaucoma patients**

**2D-PAGE**

Protein-patterns in circulating leucocytes demonstrate clear similarities between vasospasm and normal-tension glaucoma versus controls. Moreover, protein-clusters can be considered for predictive imaging of healthy vasospastic individuals to glaucoma as shown in Fig. 4.

**Expression array**

The image of hybridised “AtlasTM Human Cardiovascular Array” revealed similarities as well as alterations in expression-patterns among normal-tension glaucoma (NTG), high-tension glaucoma (HTG), and VD groups versus controls are summarised in Table 1.

Compared to the control group the expression of 146, 68, and 60 genes were found to be altered in NTG, HTG, and VD groups respectively; the same 53 genes were differentially expressed in both NTG and HTG groups versus controls. Among 146 genes differentially expressed specifically in NTG group we monitored 48 and 53 genes which were similarly expressed either in VD or in HTG groups respectively. Among 68 genes differentially expressed specifically in HTG group we found 43 genes to be similarly expressed in VD group only. The highest difference—146 genes—was found to be between NTG and control groups. In contrast, the lowest difference—21 genes—was demonstrated to be between VD and the overlap of NTG/HTG.

34 genes demonstrated similar expression alterations in NTG, HTG, and VD groups versus controls as given in Table 2. As the differentially expressed overlap VD/NTG/
HTG was compared with the control group, following most significant difference was monitored:

- P2Y purinoreceptor 7
- Na\(^+\)/Ca\(^{2+}\) exchange protein 1 (Na\(^+\)/Ca\(^{2+}\) EP1)
- Intercellular adhesion molecule 1 (ICAM1)
- The cluster of the tissue remodelling metalloproteinases.

This group of gene-transcripts is proposed to be the reliable target to design advanced diagnostic tools for predictive glaucoma diagnosis in healthy vasospastic individuals [15].

P2Y purinoreceptor is upregulated in vasospastic individuals and glaucoma patients. The movement of leucocytes...
A. Top

![Image](image1.png)

B. Bottom

![Image](image2.png)

**Fig. 4** Proteomics-imaging of blood-biomarkers (ex vivo identification in circulating leucocytes) specific for normal-tension glaucoma (NTG). A. Top: The pathology-specific protein-cluster is completely suppressed in both NTG and vasospasm in contrast to controls.

**Table 1** Numbers of genes an expression of which is either differential or equal among the groups tested as shown by “Expression array”. Thereby, 108 genes were found to be differentially expressed between NTG and HTG groups. 34 genes demonstrated similar alteration for vasospastic individuals (VD) and both glaucoma-patient groups when compared to the healthy controls (see these genes listed in Table 2).

| Differential to control | VD equal to | VD Differential to |
|-------------------------|------------|--------------------|
| NTG → 146               | Control → 528 | NTG → 109          |
| HTG → 68                | NTG → 48   | K → 60             |
| VD → 60                 | HTG → 43   | HTG → 43           |
| NTG = HTG → 53          | **NTG = HTG → 34** | NTG = HTG → 21     |

**ICAM-1 is upregulated in vasospastic individuals and glaucoma patients** Neutrophil-endothelium interactions are implicated in pathological alterations of blood vessel permeability, plasma extravasation, diapedesis of white blood cells, and their important role in adaptive immune responses as reviewed by Di Gennaro et al. [24]. Specifically, a highly enhanced concentration of leukotrienes B4 and C4 has been observed in CSF of patients with multiple sclerosis [25]. The member of leukotrienes receptors family—LTB4 receptor or P2Y purinoceptor 7—for the first time has been isolated from human erythroleukaemia cell cDNA library [26]. The stimulation of monocytes, neutrophils, and endothelial cells was suggested to be a physiological role for the LTB4 receptor [27]. There is a growing body of evidence indicating an important role of LTB4 receptors in regulation of pathologic inflammation. Particularly using animal inflammatory models a reduced disease severity has been shown as LTB4 receptor antagonists have been applied; the same effect has been observed in mice with target deletion of BLT1—a high-affinity LTB4 receptor primarily expressed in leucocytes [28]. Furthermore, some studies support a potential role of P2Y receptors in controlling intraocular pressure, although additional investigations of the issue are necessary [29].
Table 2 Differentially expressed genes (altogether 34 ones as also summarised in Table 1) versus controls, the transcriptional levels of which were similar for VD, NTG and HTG groups [18]

| Double-spot position in “EA”-image exp. difference vs. control | Name of gene as given in “Atlas™ Human Cardiovascular Array” | GenBank Accession | SwissProt Accession | Gene/Protein Classification |
|---|---|---|---|---|
| A7d increased | P2Y purinoceptor 7 (P2Y7); leukotriene B4 receptor; Chemoattractant receptor-like1 (CMKRL1) | U41070 | Q15722 | Other receptors (by Ligands) |
| | | | | G Protein-Coupled Receptors |
| A7e increased | Retinoic acid (Vitamin-A1-Säure) receptor gamma 1 (RAR-gamma 1; RARG) | M24857 | P13631 | Transcription Activator & Repressors |
| | | | | Hormone Receptors |
| | | | | Nuclear Receptors |
| B1n increased | Androgen receptor coactivator 70-kDa subunit (ARA70) | L49399 | Q13772 | Transcription Activator & Repressors |
| | | | | Symporters & Antiporters |
| B4c increased | G protein-activated inward potassium channel 4 (GIRK4); heart K+/-ATP channel (KATP1); cardiac inward rectifier (CIR); KIR3.4 | U39195 | P48544 | Voltage-gated Ion Channels |
| | | | | Q92807 |
| B4d increased | Sodium/calcium exchanger 1 precursor; Na+/Ca2+-exchange protein 1 | M91368 | P32418 | ABC transporters |
| | | | | Drug-Resistance proteins |
| | | | | Xenobiotic Transporters |
| | | | | ABC transporters |
| B4e increased | Multidrug resistance protein 3 (MDR3); P-Glycoprotein 3 (PGY3) | M23234 | P21439 | Other Inorganic Ions & Channels |
| | | | | Other Inorganic Ions & Channels |
| B5f increased | Endothelial nitric oxide synthase (EC-NOS) | M93718 | P29474 | Other Metabolism Enzymes |
| | | | | Other Intracellular Transducers, Effectors & Modulators |
| B6d increased | Intercellular adhesion molecule 1 precursor (ICAM1); major group rhinovirus receptor; CD54 antigen | J03132 | P05362 | Matrix Adhesion Receptors |
| B7g increased | Calcium & integrin-binding protein (CIB) | U85611 | Q99828 | Calcium-Binding proteins |
| C1g increased | Cadherin 7 (CDH7) | AF047826 | O60574 | Cell Surface Antigens |
| | | | | Cell-Cell Adhesion Receptors |
| C1h increased | Intestinal peptide-associated transporter 1 (HPT1) | U07969 | Q12864 | Other Cell Adhesion proteins |
| | | | | Other Cell Adhesion Proteins |
| | | | | Other Facilitated Diffusion proteins |
| C2i increased | GAP junction alpha-5 protein | L34954 | P36382 | Cell-Cell Adhesion Receptors |
| | | | | Other Membrane Channels & Transporters |
| C3 m increased | Integrin beta 2 (ITGB2); cell surface adhesion glycoproteins LFA-1/CR3/p150, 95 beta subunit precursor; CD18 antigen; Complement receptor C3 beta subunit | M15395 | P05107 | Cell-Cell Adhesion Receptors |
| D1 m increased | Cardiac LIM domain protein; muscle LIM protein; cystein-rich protein 3 (CRP3); LIM-only protein 4 | U49837 | P50461 | Basic Transcription Factors |
| D1n increased | Cardiotrophin-1 (CT1) | U43030 | Q16619 | Growth Factors, cytokines & Chemokines |
| D2n increased | Matrix metalloproteinas 16 (MMP-16) | D83646 | P51512 | Chromatin Proteins Metalloproteinas |
| Double-spot position in “EA”-image exp. difference vs. control | Name of gene as given in “Atlas™ Human Cardiovascular Array” | GenBank Accession | SwissProt Accession | Gene/Protein Classification |
|---------------------------------------------------------------|---------------------------------------------------------------|-------------------|-------------------|----------------------------|
| D4a increased                                                 | TIMP-3                                                         | U14394            | P35625            | Extracellular Matrix Proteins |
|                                                               |                                                                |                   |                   | Proteinase Inhibitor        |
| D4b increased                                                 | TIMP-4                                                         | U76456            | Q99727            | Extracellular Matrix Proteins |
|                                                               |                                                                |                   |                   | Proteinase Inhibitor        |
| D4d increased                                                 | Sterol regulatory element-binding transcription factor 1       | U00968            | P36956            | Basic transcription Factors |
|                                                               |                                                                |                   |                   | Other Apoptosis-Associated Proteins |
| D4e increased                                                 | Sterol regulatory element-binding transcription factor 2       | U02031            | Q12772            | Basic transcription Factors |
|                                                               |                                                                |                   |                   | Other Apoptosis-Associated Proteins |
| D5 increased                                                 | Rab geranylgeranyl transferase bety subunit                    | Y08201            | P53611            | Trafficking & Targeting Proteins |
|                                                               |                                                                |                   | Q92697            | Protein Modification Enzymes |
|                                                               |                                                                |                   |                   | GTP/GDP Exchangers & GTPase Activity Modulators |
|                                                               |                                                                  |                   |                   | DNA Synthesis, Recombination & Repair Proteins |
|                                                               |                                                                |                   | L40817            | Apoptosis-Associated Proteins |
|                                                               |                                                                  |                   |                   |                                |
| D6 decreased                                                 | Muscle-specific DNase 1-like precursor (DNase 1 L1; DNL 1 L); Dnase X | X90392            | P49184            | Complex Lipid Metabolism      |
|                                                               |                                                                |                   |                   | Xenobic Metabolism            |
|                                                               |                                                                |                   |                   |                                |
| E1b increased                                                 | Lanosterol synthase (LSS); oxidosqualene lanosterol cyclase (OSC) | U06846            |                   | Complex Lipid Metabolism      |
|                                                               |                                                                |                   |                   |                                |
| E3n increased                                                 | NADPH-cytochrome p450 reductase                                 | S90469            | Q16455            | Complex Lipid Metabolism      |
|                                                               |                                                                |                   | P16435            |                                |
|                                                               |                                                                |                   |                   | Xenobic Metabolism            |
|                                                               |                                                                |                   |                   |                                |
| E4 increased                                                 | Steroid 5 alpha reductase 1 (SRD5A1); 3-oxo-5-alpha steroid 4 dehydrogenase 1 | M32313            | M68886            | Complex Lipid Metabolism      |
|                                                               |                                                                |                   | M74047            | Complex Lipid Metabolism      |
|                                                               |                                                                |                   | M68886            |                                |
|                                                               |                                                                |                   |                   | DNA Synthesis, Recombination & Repair Proteins |
|                                                               |                                                                |                   | L40817            |                                |
|                                                               |                                                                |                   |                   | Apoptosis-Associated Proteins |
|                                                               |                                                                  |                   |                   |                                |
| F2d increased                                                 | Pregnane X receptor (PXR)                                      | AF061056          | O75469            | Hormone receptors            |
|                                                               |                                                                |                   |                   | Nuclear Receptors             |
| F2e increased                                                 | Estrogen-related receptor gamma                                | AF058291          | O75454            | Hormone receptors            |
|                                                               |                                                                |                   |                   | Nuclear Receptors             |
| F2f increased                                                 | Nuclear receptor subfamily 4 group A member 2 (NR4A2); nuclear receptor-related protein 1 (NURR1); transcriptionally inducible nuclear receptor (TINUR); NOT | X75918            | P43354            | Hormone Receptors            |
|                                                               |                                                                |                   |                   | Nuclear Receptors             |
|                                                               |                                                                |                   |                   | Transcription Activators & Repressors |
| F2i increased                                                 | Orphan nuclear receptor TR4; nuclear receptor subfamily 2 group c member 2 (NR2C2); TAK1 | U10990            | P55092            | Orphan Receptors             |
|                                                               |                                                                |                   | P49116            | Nuclear Receptors             |
|                                                               |                                                                |                   |                   | Transcription Activators & Repressors |
| F3a increased                                                 | RAR-related orphan receptor C                                   | U16997            | P51449            | Orphan Receptors             |
|                                                               |                                                                |                   |                   | Nuclear Receptors             |
|                                                               |                                                                |                   |                   | Transcription Activators & Repressors |
| F3e increased                                                 | LX receptor alpha (LXR alpha)                                   | U22662            | Q13133            | Orphan Receptors             |
|                                                               |                                                                |                   |                   |                                |
function, potentially leading to circulatory disturbances [30]. Interactions between blood cells and the vessel wall result in endothelial dysfunction and injury leading to increased blood-brain barrier permeability and even oedema formation [31]. Penetration of leucocyte into inflamed areas involves complex interaction of leucocytes with endothelium through regulated expression of surface adhesion molecules. Found in this work to be highly expressed in VD, NTG and HTG groups ICAM-1 is believed to be largely responsible for the adhesion and trans-endothelial migration of leucocytes [32]. This is well in agreement with earlier developed strategies aimed at inhibition of endothelial interactions with leucocytes via use of adhesion molecule monoclonal antibodies, which successfully reduce cerebral ischemia/reperfusion injury, infarct size, and demonstrate a neuroprotective effect generally [33–35]. In our study, highly expressed ICAM-1 was found in leucocytes of glaucoma patients; in contrast, if any only traces of the target expression was detected in the leucocytes of healthy controls.

Sodium calcium exchanger (NCE) Many studies examined the levels of cytosolic Ca$^{2+}$ ([Ca$^{2+}$]$_c$) and Na$^+$ ([Na$^+$]) in human blood cells, whereby leucocytes have been the main target of studying the relationship between blood pressure and intracellular content of both ions as reviewed by Horiguchi et al. [36]. As it has been shown by Horiguchi et al., the resting [Ca$^{2+}$]$_c$ correlates well with NCE expression indicating NCE expression regulation to be an adaptive mechanism for Ca$^{2+}$ extrusion mediation. The same study observed also a gender effect on [Ca$^{2+}$]$_c$/[Na$^+$] regulation in circulating leucocytes being in relationship with blood pressure. Further, the role of endothelial intracellular Ca$^{2+}$ concentration in molecular mechanisms of vasoconstriction/vasodilatation has been intensively studied, and the functional association between P2Y purinoceptors, endothelial NO synthesis and calcium transport in terms of vascular regulation is well documented in the literature [37, 38]. Our findings here clearly demonstrate the up-regulation of both P2Y purinoceptor and Na$^+$/Ca$^{2+}$ exchanger in circulating leucocytes of glaucoma patients as well as vasospastic individuals versus healthy controls.

Tissue remodelling metalloproteinases Significantly increased protein expression rates of both latent and active forms of metalloproteinases MMP-9 and MT1-MMP in circulating leucocytes correlate well with the enhanced levels of transcription and with glaucoma diagnosis [12]. Once activated, both hydrolases necessarily contribute to remodelling or even degeneration of the tissue whereto they are secreted by circulating leucocytes. This up-regulation might be a consequence of repeated mild ischemia/reperfusion postulated for both vasospastic individuals and glaucoma patients [18]. However, the question as to whether or not there is a correlation between an increased MMPs activity and glaucoma severity should be further clarified.

Furthermore, the increased synthesis of tissue-remodelling hydrolases detected in blood of healthy vasospastic individuals can potentially lead to development of some other pathologies which have not been considered as related to vasospasm till now. To the potential list of them belong altered wound-healing, some types of organ-degeneration, increased metastases activity. Large-scale studies are essential to be preformed, in order to prove a potential impact of vasospasm for the above listed pathophysiologic processes / manifested pathologies. This allows a targeted prevention at the stage of pre-lesions, “upstream” disease manifestation.

Concluding remarks

- Expression similarities between glaucoma and VD versus controls indicate, on one side, a predisposition of VD individuals to glaucomatous damage, and, on the other side, an important role of vascular component in glaucoma pathology.
- Expression dissimilarities between VD and glaucoma patients might indicate some glaucoma-specific patho-
molecular rearrangement in leucocytes of both VD and EPMA Journal (2010) 1:263

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