Vascular endothelial sampling and analysis of gene transcripts: a new quantitative approach to monitor vascular inflammation

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Onat D, Jelic S, Schmidt AM, Pile-Spellman J, Homma S, Padeletti M, Jin Z, Jemtel TH, Colombo PC, Feng L. Vascular endothelial sampling and analysis of gene transcripts: a new quantitative approach to monitor vascular inflammation. J Appl Physiol 103: 1873–1878, 2007. First published August 23, 2007; doi:10.1152/japplphysiol.00367.2007.—Background: Limited access to endothelial tissue is a major constraint when investigating the cellular mechanisms of vascular inflammation in patients with cardiovascular and metabolic diseases. We introduce venous endothelial sampling coupled to quantitative analysis of gene transcripts by real-time PCR, as a novel approach to study endothelial gene expression in human subjects. Methods: Endothelial cells were collected from a superficial forearm vein using five guide wires sequentially inserted through a 20-gauge angiocatheter in seven patients with history of cardiovascular events related to advanced vascular disease and in 17 healthy subjects. Endothelial cells were purified using magnetic beads coated with endothelial specific antibodies. Endothelial mRNA was amplified using RiboAmp HS RNA Amplification kit (Molecular Devices, Sunnyvale, CA). Amplified RNA was analyzed by real-time PCR. Results: Linearity of RNA amplification was validated by real-time PCR using RNA from 1,000 human umbilical endothelial cells (HUVECs) before and after amplification. In human subjects, vascular disease was associated with significant induction of proatherosclerotic genes: early growth response gene product (Egr-1) and monocyte chemoattractant protein-1 (MCP-1). Conclusion: Venous endothelial sampling coupled to real-time PCR analysis is a minimally invasive, safe, and reliable technique to monitor vascular inflammation in human subjects. Expression of genes implicated in the atherosclerotic process is increased in the venous endothelium of patients with arterial vascular disease. Venous endothelial sampling and quantitative analysis of gene expression may help develop new vascular-targeted biomarkers to identify and track the impact of disease states and therapeutic interventions in vascular diseases.

inflammation; vein; vascular biology

ENDOTHELIAL CELLS MODULATE many important vascular functions, such as control of vascular tone, inflammation, hemostasis, and angiogenesis (21). Despite its importance in the pathogenesis of atherosclerosis, the study of vascular inflammation has been hampered by the lack of adequate techniques to sample and characterize the human endothelium. In the present study we describe a minimally invasive technique of venous endothelial sampling coupled to analysis of gene transcripts by real-time PCR and demonstrate that this in vivo approach can be used to quantitatively measure endothelial gene expression in human subjects. This method is safe and allows repetitive sampling of endothelial cells from an accessible vascular segment. We previously used a qualitative PCR method to show that proatherosclerotic genes, including early growth response gene product (Egr-1) and monocyte chemoattractant protein-1 (MCP-1), are induced in the arterial endothelium of diabetic patients undergoing cardiac catheterization (8, 9). Here we present our preliminary data on using venous endothelial sampling and quantitative real-time PCR analysis to measure endothelial Egr-1 and MCP-1 gene expression in patients with history of advanced vascular disease compared with healthy subjects. Venous endothelial sampling coupled to real-time PCR analysis provides a new approach to study vascular inflammation in patients with cardiovascular and metabolic diseases.

MATERIALS AND METHODS

Human subjects. Patients were eligible for the study if they had advanced vascular disease, as defined by a history of at least one of the following cardiovascular events: acute myocardial infarction, nonembolic/non-hemorrhagic stroke or transient ischemic attack, limb ischemia (i.e., claudication or amputation), or any revascularization procedures that occurred more than 30 days prior to enrollment. Patients with chronic renal insufficiency (serum creatinine >3 mg/dl), chronic or intermittent therapy with steroids, cancer, chronic inflammatory conditions, pregnancy, nursing were excluded from the study.

Healthy subjects had to be 18 years old or older, nonsmokers, not hypertensive or diabetic, and not receiving regular medical therapies. All subjects signed an informed consent. The study protocol and the informed consent were approved by the local Institutional Review Board.

Venous endothelial sampling. Venous endothelial cells were collected as previously described (3, 4). A 20-gauge angiocatheter was inserted into a superficial arm vein. Five 0.018-in. diameter J-shaped endovascular wires (Arrow, Reading, PA) were sequentially inserted through the angiocatheter and advanced for ~10 cm to dislodge endothelial cells and trap them on the serrated surface of the wire. Wire tips were cut approximately at 4 cm and inserted into plastic tubing (length 5 cm, inner diameter 0.65 in.; Hospira Lake Forest, IL). The tubing was distally connected to two sterile syringes (3 ml) (Becton Dickinson, Franklin Lakes, NJ), which were previously loaded with sterile endothelial dissociation buffer (EDB) (0.5× PBS (without CaCl2 or MgCl2), 0.5% Hanks’ solution (without CaCl2, MgSO4, MgCl2, sodium bicarbonate, or phenol red), 0.5% bovine albumin fraction V, 2 mM EDTA (Invitrogen, Carlsbad, CA), 200 μg/ml heparin sodium Salt (Sigma, St. Louis, MO)) and 1 U/μl SUPERase-In RNase inhibitor (Ambion, Austin, TX). Air was care-
Purified cells were resuspended in 10 l lysates instead of isolated RNA (data not shown). For that purpose, RNeasy Micro kit (Qiagen, Valencia, CA) and PicoPure RNA Isolation Markers to confirm the reliability of this purification step, as previously documented in hyperglycemic (1) and hyperlipidemic states (13), which are associated with atherosclerosis. Real-time PCR primer and probe sequences are listed in Table 1. In addition exon-exon boundary spanning receptor for advanced glycation end products (RAGE) primers (Applied Biosystems; GenBank accession number: NM_001136, Catalogue number: Hs00542592_g1) were used to confirm the absence of contamination from genomic DNA in aRNA samples. For real-time PCR reaction the following reagents were added: 1.25 µl cDNA solution + 12.5 µl TaqMan master mix (2X) + primers and probes for MCP-1, Egr-1, or β-actin, and nuclease-free water up to 25 µl total. This reaction mix was added to the wells as triplicates for each sample. Samples were considered acceptable for analysis when the cycle threshold (Ct) value for β-actin was <30 cycles. An outline of the steps leading from endothelial sampling to real-time PCR analysis is detailed in Fig. 2.

**Cell culture.** Human umbilical vein endothelial cells (HUVECs) were isolated and cultured, as previously reported (3), until second passage in standard culture media (control): Medium 199 (M199) with glutamine containing 20% heat inactivated newborn calf serum, 50% fully removed from the system. The cells were collected from each individual wire by washing inside the tubing and then transferred to Eppendorf tubes for the endothelial purification step (see below). Potential complications (e.g., pain, phlebitis, infection, thrombosis) were assessed by history and physical examination at 1-wk clinical follow up.

**Endothelial cell purification and preparation of cell lysates.** Endothelial cells from the samples were purified using magnetic beads (Dynal, Lake Success, NY) coated with anti-CD146 antibody (Chemicon, Temecula, CA), a mouse monoclonal antibody specific for endothelial cells, as previously described (8). Endothelial cells purification was performed at 4°C to avoid alteration of endothelial phenotype. All experiments were carried out under a Benchtop UV Sterilization PCR Workstation to protect against environmental contamination. For the same reason, only filtered tip and individually wrapped RNase-DNase-free Eppendorf tubes were used. Routine quality control assessment was performed by reverse transcription PCR (RT-PCR) using endothelial, smooth muscle, and leucocyte markers to confirm the reliability of this purification step, as previously described (8). Two examples of the PCR products from a purified endothelial sample are shown in Fig. 1.

In preliminary studies, RNA isolation from purified cells using RNeasy Micro kit (Qiagen, Valencia, CA) and PicoPure RNA Isolation Kit (Molecular Devices, Sunnyvale, CA) resulted in insufficient RNA yield. Our yield improved by proceeding directly from cell lysates instead of isolated RNA (data not shown). For that purpose, purified cells were resuspended in 10 l lysis buffer (Ambion) and incubated at 75°C for 10 min. Samples were cooled to room temperature. Genomic DNA was degraded by incubation with 2 U/µl DNase I (Ambion) at 37°C for 15 min to prevent genomic DNA contamination. The reaction was terminated by incubating samples at 75°C for 5 min. Lysed cells were stored at -80°C or further carried on to RNA amplification.

**RNA amplification.** Because of the minute amount of RNA in the cell lysates, RNA isolation with highly efficient method, such as RNeasy Micro kit (Qiagen, Valencia, CA) or PicoPure RNA Isolation Kit (Molecular Devices), still resulted in the loss of most of the RNA, precluding real-time PCR analysis. The endothelial cell lysates were therefore subjected to linear RNA amplification using RiboAmp HS RNA Amplification Kit (Molecular Devices) before real-time PCR analysis. RNA amplification was otherwise carried out according to the manufacturer’s instruction. Oligo(dT) primers were used to selectively amplify mRNA during the first round of RNA amplification. The quantity and quality of the amplified antisense RNA (aRNA) was assessed by spectrophotometer and Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA), respectively.

**RT-PCR.** Superscript III First Strand Synthesis System for RT-PCR (Invitrogen) was used to obtain cDNA from aRNA. In preliminary studies, different primer combinations/relative ratios of Oligo(dT)20, Oligo(dA)20, and Random hexamers were tested: a combination of 50 µM Oligo(dA)20 and 50 ng/µl random hexamers resulted in the best cDNA yield. The primers and 2 µg of aRNA (the same quantity of RNA was used in all reactions) were incubated together for 10 min at 25°C, then for 50 min at 50°C. Otherwise the reaction was carried out as indicated by the manufacturer. Control experiments with no reverse transcription were also run to rigorously assess the efficacy of the DNase treatment.

**Real-time PCR.** Quantitative real-time PCR was performed using TaqMan method on ABI Prism 7900 HT Sequence Detection System (Perkin Elmer). TaqMan primers and probes for β-actin, Egr-1, and MCP-1 were designed using Primer Express Software (Perkin Elmer) to fall into the 500 bp of 3’ region of mRNA. Their relative efficiency was validated using 10× serially diluted RNA (range = 100–0.01 ng) pooled from human mammary gland PolyA+RNA, human lung PolyA+RNA, human spleen PolyA+RNA, and human heart PolyA+RNA (BD Biosciences, Palo Alto, CA), which are known to express high levels of MCP-1 and Egr-1 transcripts (data not shown).

Real-time PCR products were sequenced to confirm the specificity of real-time PCR analysis using the AmpliCycle sequencing kit (Perkin Elmer). β-Actin was selected as an endogenous control due to its documented stability in hyperglycemic (1) and hyperlipidemic states (13), which are associated with atherosclerosis. Real-time PCR primer and probe sequences are listed in Table 1. In addition exon-exon boundary spanning receptor for advanced glycation end products (RAGE) primers (Applied Biosystems; GenBank accession number: NM_001136, Catalogue number: Hs00542592_g1) were used to confirm the absence of contamination from genomic DNA in aRNA samples. For real-time PCR reaction the following reagents were added: 1.25 µl cDNA solution + 12.5 µl TaqMan master mix (2X) + primers and probes for MCP-1, Egr-1, or β-actin, and nuclease-free water up to 25 µl total. This reaction mix was added to the wells as triplicates for each sample. Samples were considered acceptable for analysis when the cycle threshold (Ct) value for β-actin was <30 cycles. An outline of the steps leading from endothelial sampling to real-time PCR analysis is detailed in Fig. 2.

**Table 1. Real-time PCR primer and probe sequences**

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>Real-Time PCR Primer and Probe Sequences 5'→3'</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>Fwd: CTG GAA CAA TGG TGA AGG TGA CA</td>
<td>NM_001101</td>
</tr>
<tr>
<td></td>
<td>Rev: CCG CCA CAT TGT GAA CTT CTG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: VIC-TGC TGC GTC CAA CAA CCG-MGBNFQ</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>Fwd: CAC AGG TGC CTT CAG GAT GGC A</td>
<td>NC_000017</td>
</tr>
<tr>
<td></td>
<td>Rev: AGT GAG TCT TCA AGT CTT CGG AGT T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 6FAM-CAC CTG CAG GAC GAA CAA-MGBNFQ</td>
<td></td>
</tr>
<tr>
<td>Egr-1</td>
<td>Fwd: AGT TTC AAC TCT TGG TGC CTT T</td>
<td>NM_001964</td>
</tr>
<tr>
<td></td>
<td>Rev: CGC TCA CAA TGG CAC ATG TCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe: 6FAM-CAC CAA GCC GCA TCA-MGBNFQ</td>
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MCP-1, monocyte chemoattractant protein; Egr-1, early growth response gene product.

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Innovative Methodology

**Gene Expression in Human Vascular Endothelium**

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**Fig. 1.** Expression of cell markers in patient-derived samples after endothelial cell purification. Two examples (1, 2) of PCR results of patient-derived endothelial cells are shown. The internal control GAPDH, is the top band on the left. Two endothelial markers, thrombomodulin and von Willebrand factor are the bottom bands on the left and in the center of the figure, respectively. Smooth muscle marker, α-actin, or leucocyte marker leucocyte common antigen-1 (LCA-1), were not detected. M, marker.
GENE EXPRESSION IN HUMAN VASCULAR ENDOTHELIUM

RESULTS

Validation of quantitative real-time PCR analysis of amplified RNA. Egr-1, MCP-1, β-actin real-time PCR products were detected in aRNA even in the absence of RT. The cycle difference between RT+ vs. RT− products was 11.51 ± 0.24 for Egr-1, 9.26 ± 1.41 for MCP-1, and 9.88 ± 0.78 for β-actin. This minor DNA contamination despite DNase treatments (ranging 1/600 to 1/3,000 of total cDNA after RT) could result either from incomplete DNA degradation of genomic DNA or from incomplete DNA degradation of the large amount of cDNA generated during RNA amplification. The first possibility was excluded by testing exon-exon boundary spanning RAGE primers on the same amplified RNA samples. Real-time PCR products were detected even in the absence of RT with a cycle difference of 7.81 ± 1.30 between RT+ vs. RT− products. Therefore DNA contamination resulted from incomplete degradation of the cDNA generated during RNA amplification. Overall, these data indicate that after RNA amplification, DNA contamination is present but, otherwise, is quite limited, representing, after RT, <1/600 of total Egr-1, MCP-1, and β-actin cDNA.

Linearity of RNA amplification for Egr-1 was assessed by real-time PCR using RNA from HUVECs before and after amplification (Fig. 3A). The relative expression (ΔCt) of Egr-1 to β-actin was 9.69 ± 1.85 (also equal to 2.20 ± 2.31 × 10−3 relative copy number) before, and 9.0 ± 0.70 after RNA amplification (also equal to 2.12 ± 0.90 × 10−3 relative copy number) with a cycle difference between pre- and postamplification of only 0.69 cycles. The Wilcoxon signed-rank statistic

![Diagram](http://jap.physiology.org/)
confirmed the agreement between pre- and postamplification analyses \( (P = 0.63) \).

Linearity of RNA amplification for MCP-1 was assessed by real-time PCR using RNA from HUVECs before and after amplification (Fig. 3B). The relative expression (ACT) of MCP-1 to \( \beta \)-actin was 6.31 ± 1.60 before (also equal to 1.30 ± 0.14 \times 10^{-2} \) relative copy number) and 9.88 ± 0.04 (also equal to 1.06 ± 0.03 \times 10^{-2} \) relative copy number) after amplification, thus showing an amplification bias favoring \( \beta \)-actin over MCP-1. However, this bias was fixed at approximately three cycles, regardless of the concentration of MCP-1 in the sample. In HUVECs treated with LPS, the relative expression (ACT) of MCP-1 to \( \beta \)-actin was 1.12 ± 0.21 before (also equal to 4.63 ± 0.65 \times 10^{-1} \) relative copy number), and 4.46 ± 0.04 (also equal to 4.54 ± 0.13 \times 10^{-2} \) relative copy number) after amplification. Because of this fixed amplification bias, the calculated difference in MCP-1 expression between control HUVECs and HUVECs treated with LPS was equal to 5.19 cycles (ACT - ACTC) before amplification, and 5.42 cycles (ACT - ACTC) after amplification. Considering that MCP-1 induction with LPS treatment in HUVECs is more than 30 folds, this small difference of 0.23 cycles is negligible \( (P = 0.50 \) by Wilcoxon signed-rank statistic). Similar results were obtained when the initial HUVECs input was reduced from 1,000 to 200 cells.

Linear RNA amplification can thus be used to increase the sensitivity of real-time PCR, yet maintaining the relative ratios of gene expression.

MCP-1 and Egr-1 expression in the venous endothelium of patients with vascular disease and of healthy subjects. Seven patients (5 men, mean age 56 ± 12 yr) with history of cardiovascular events (4 with history of myocardial infarction, and 3 with history of limb ischemia requiring distal limb amputation in 2 of them), and 17 healthy subjects (8 men, mean age 39 ± 11 yr) were studied. Patients were older than healthy subjects \( (P < 0.05) \). All patients were diagnosed with Type 2 diabetes mellitus. Five patients were treated with insulin and two with oral hypoglycemic drugs. All patients also had a history of hypercholesterolemia and received treatment with a statin. Four of seven (57%) also had a history of hypertension. Four of seven (57%) were treated with angiotensin converting enzyme inhibitors. All patients received antiplatelet treatment.

All endothelial samples were successfully and purely amplified. There was no contamination of white blood cells or smooth muscle cells. Total aRNA ranged from 15 to 24 \( \mu \)g. Six of seven (86%) patients with advanced vascular disease and 14 of 17 (82%) healthy samples were acceptable for analysis based on the criteria defined above in MATERIALS AND METHODS. Overall, Ct value for \( \beta \)-actin averaged 25.30 ± 3.20 cycles (25.12 ± 3.00 cycles in healthy subjects and 25.82 ± 4.41 cycles in patients). The time for endothelial sampling and purification (i.e., from the sampling to the lysing of purified endothelial cells) averaged 54 ± 8 min.

Endothelial Egr-1 expression was 30-fold higher in patients with advanced vascular disease than in healthy subjects \( (0.66 ± 1.22 \times 10^{-2} \) vs. 2.18 ± 2.82 \times 10^{-4}, \) relative copy number, \( P = 0.06; \) Fig. 4A). Endothelial MCP-1 expression was fivefold and significantly higher in patients than in healthy subjects \( (4.65 ± 5.32 \times 10^{-3} \) vs. 1.00 ± 2.11 \times 10^{-3} \) relative copy number, \( P < 0.05; \) Fig. 4B).

We also categorized patients and healthy subjects into two groups based on levels of Egr-1 and MCP-1 expression, using a \( \Delta C T < 14 \) (corresponding to a relative copy number of \(< 6.10 \times 10^{-5} \) as an arbitrary cut-off value. In healthy individuals, lower levels of Egr-1 and MCP-1 expression were detected in 8/14 (57%) and in 6/14 (43%) of the subjects respectively, whereas none of the vascular disease patients had lower levels of Egr-1 \( (P < 0.05) \) and MCP-1 expression \( (P = 0.05) \).

The procedure of venous endothelium sampling was found to be safe. At 1-wk follow-up, only one patient of the total 24 included in this study developed superficial phlebitis. This phlebitis was painful, but otherwise benign in its course, requiring only treatment with non-steroidal anti-inflammatory drugs for 2 days.

**DISCUSSION**

Safe and minimally invasive collection of vascular endothelial cells coupled to measurements of gene expression by

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**Fig. 4.** Egr-1 and MCP-1 gene expression in venous endothelial cells. Quantitative analysis of endothelial Egr-1 (A) and MCP-1 (B) mRNA was performed in venous endothelial cells collected from patients with advanced vascular disease \( (n = 6) \) and from healthy subjects \( (n = 14) \). Egr-1 and MCP-1 gene expression were higher in vascular disease patients than in healthy subjects \( (P = 0.06 \) and \( P < 0.05 \), respectively). Individual \( (\bullet) \) and mean \( (\text{bar}) \) values are indicated as relative copy number of Egr-1 and MCP-1 to \( \beta \)-actin (endogenous control).

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quantitative real-time PCR analysis, provides a novel and unique approach to study the vascular endothelium in human subjects. Evaluation of vascular endothelial dysfunction in patients with metabolic and cardiovascular diseases has been, so far, mostly limited to the assessment of the nitric oxide-mediated control of the vascular tone. We previously validated and, together with other investigators, applied a quantitative method to measure endothelial protein expression using immunofluorescent microscopy (3, 4, 6, 9, 19). Quantitative real-time PCR analysis of endothelial mRNA further expands our insight into the molecular events that alter the vascular endothelium and, differently from protein analysis, makes it possible to measure within hours the endothelial response to acute interventions.

The method of linear RNA amplification described in this study allows reliable measurement of multiple transcripts in a very small number of patient-derived endothelial cells. The linearity of RNA amplification was confirmed by real-time PCR using RNA from primary cultured endothelial cells. Although the degree of amplification varies slightly for different genes, the relative ratio of gene expression is well maintained. Therefore, this technique is well suited to simultaneously monitor over time the expression of many endothelial genes relative to an internal control.

Experimental evidence has implicated Egr-1 and MCP-1 as pivotal mediators of atherosclerosis (5, 8, 11, 12, 16, 17, 20). Egr-1, induced by hyperglycemia, vascular injury, and hypoxia, is a zinc finger transcription factor that promotes expression of key inflammatory mediators and growth factors (11). Mice deficient in Egr-1 display decreased atherosclerosis and vascular inflammation in atherosclerosis-prone apolipoprotein E-deficient mice (11). MCP-1 is a chemokine whose expression early in atherosclerosis is critical for monocyte adhesion to the endothelium (5, 16, 17).

Molecular analysis of the venous endothelium using vascular endothelial sampling coupled to quantitative analysis of gene transcripts by real-time PCR indicates that expression of Egr-1 and MCP-1 are increased in patients with advanced vascular disease compared with healthy subjects. However, our results must be interpreted with caution. In our study, patients were older, had diabetes and hyperlipidemia, and most of them had history of hypertension. In addition, they were all aggressively treated for their cardiovascular and metabolic conditions. The small number of subjects enrolled in this study does not allow identifying a significant association between these clinical variables and endothelial Egr-1 and MCP-1 expression. However, the main objectives of this study were 1) to determine whether gene expression can be reliably quantified in a small number of endothelial cells collected by endovascular sampling, and 2) to determine whether expression of proatherosclerotic genes is increased in the venous endothelium of patients with arterial vascular disease. Age, diabetes, hyperlipidemia, hypertension, and drug treatment might all have contributed to alter the endothelial phenotype in our patients with advanced vascular disease (7). Additional studies with larger cohorts of subjects will be necessary to establish a link between these clinical variables on one side and endothelial Egr-1 and MCP-1 expression on the other.

Mechanical manipulation of endothelial cells during sampling and purification is another potential confounder that may have altered the endothelial phenotype. However, duration of these steps was similar (~50 min) in samples from patients and healthy volunteers, thus minimizing the variance.

One potentially meaningful observation of this study relates to the wide range of endothelial Egr-1 and MCP-1 expression that we observed within each group of patients and healthy subjects (Fig. 4). This finding may suggest that vascular disease is properly treated in some patients, but not in others, and that some healthy subjects are not as “healthy” as one may think. Prospective studies may establish whether a higher level of venous expression of proatherosclerotic genes predicts a worse outcome. If so, clinicians could be more aggressive with traditional therapies in these patients, and trials of specific pharmacologic therapies, suggested by individual endothelial profiling, could be planned.

This study confirms the safety and feasibility of venous endothelial sampling in an ambulatory outpatient setting. Sampling of sites of atherosclerosis in the arterial compartment carries higher risks (22) and thus will have limited role in the longitudinal monitoring of endothelial gene expression in patients with vascular diseases. Although it is not subjected to the same hemodynamics of the arterial compartment, which are necessary for the development of atherosclerosis, the venous vasculature is chronically exposed to the same circulating levels of proinflammatory factors (e.g., glucose, lipids, cytokines) and drugs that modulate the various functions of endothelial cells. For example, hyperglycemia alters structure and function of arteries and veins (2, 15). Correction of metabolic derangement is thus expected to influence not only the arterial, but also the venous endothelial phenotype as well. In addition, recent studies reported a highly significant positive correlation between nitrotyrosine formation (an intracellular marker of oxidative stress) and expression of other proteins in endothelial cells obtained from venous samples compared with arterial samples (6, 19). Analysis of endothelial gene expression profile in veins may therefore shed light on important components of the atherosclerotic process that are frequent targets of therapeutic interventions.

In conclusion, we introduced and validated a novel approach to accurately measure gene transcripts in venous endothelial cells collected from human subjects. Our results suggest that the expression of genes implicated in the atherosclerotic process is increased in the venous endothelium of patients with advanced vascular disease. Novel vascular-targeted biomarkers may help tracking the vascular impact of cardiovascular and metabolic diseases. As a frank clinical event, such as myocardial infarction, stroke, or even death, reflects the highly advanced or end stages of vascular disease, gene expression profiling of venous endothelial cells has the potential to identify key and accessible biomarkers to predict the likelihood of such event and to monitor the impact of therapeutic interventions.

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GRANTS

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Innovative Methodology

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REFERENCES


