

POST-TRANSCRIPTIONAL MODIFICATIONS OF VEGF-A mRNA IN NON-ISCHEMIC DILATED CARDIOMYOPATHY

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Abstract: Vascular endothelial growth factor (VEGF-A) is one of the most important proangiogenic factors. It has many isoforms encoded by one gene. The occurrence of these isoforms is associated with the process of alternative splicing of mRNA. Some of the splice forms are perceived as tissue specific. The aim of this study was to determine the alternative splicing of VEGF-A mRNA in dilated cardiomyopathy, especially at the level of particular myocardial layers. The assessment of post-transcriptional modifications of VEGF-A mRNA was made on specimens taken from the explanted hearts of patients undergoing cardiac transplantation. Molecular and histopathological studies were performed on particular layers of the myocardial muscle (endocardium, myocardium, epicardium). A molecular analysis of cardiac samples was performed by quantitative analysis of the mRNA of the studied VEGF-A isoforms (VEGF121, -145, -165, -183, -189, and -206) using QRT-PCR with an ABI-PRISM 7700-TaqMan sequence detector. 72 cardiac specimens taken from the explanted hearts were analyzed. Each of the studied

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Abbreviations used: DCM – dilated cardiomyopathy, IVS – interventricular septum, VEGF – vascular endothelial growth factor

VEGF-A splice forms was present in the evaluated hearts, but the types of alternative splicing of mRNA were different in particular layers. Quantitative analysis revealed different amounts of the studied isoforms. Generally, significantly increased expression of the VEGF-A isoforms was observed in samples taken from hearts with post-inflammatory etiology of cardiomyopathy.

Our conclusions are: 1. All the studied VEGF-A isoforms were found in the human hearts, including those thusfar considered characteristic for other tissues. 2. Significant differences were observed in the expression of the VEGF-A splice forms with respect to the myocardial layers and the location of the cardiac biopsy. 3. Repetitive and comparable results for samples with post-inflammatory etiology were obtained, and they revealed considerably higher amounts of VEGF-A isoforms compared to specimens with idiopathic etiology.

Key words: Vascular endothelial growth factor (VEGF), Alternative splicing, Angiogenesis, Dilated cardiomyopathy, Transcriptional activity

INTRODUCTION

Continuous progress in the biomedical sciences is essential in finding new and improving existing diagnostic and therapeutic methods. A deeper understanding of the pathomechanisms of many diseases allows physicians to treat patients more efficiently. Considerable hope has been invested in angiogenesis, the process of formation and development of new blood vessels from the native vasculature. One of the most important proangiogenic factors is vascular endothelial growth factor (VEGF-A). VEGF actually refers to a whole family of glycoprotein growth factors (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placenta growth factor – PlGF), which have structural homology, but significant differences in their biological activities [1-5]. VEGF-A is the most commonly used in experimental protocols. It has at least seven isoforms, which differ in their number of amino acid residues: VEGF121, VEGF145, VEGF148, VEGF165, VEGF183, VEGF189, VEGF206 [1, 3, 4, 6]. All the VEGF-A isoforms are encoded by one gene, assigned to chromosome 6p21.3 [7]. This gene consists of eight exons separated by seven introns. The presence of many VEGF-A isoforms is the consequence of an alternative splicing of mRNA, which is a post-transcriptional modification [1, 2, 4, 6]. The essence of this complicated and still unclear process is the presence of multiple splicing variants of the eight exons after the seven introns have been enzymatically cut out, due to some exons being passed over in the process of final combination. The lack of particular exons leads to the change in the biological activities of particular splice forms. It is known that exons 6 and 7 are responsible for encoding two independent domains binding heparin [1-4]. Thus, the shortest isoform, VEGF121, which lacks fragments encoded by those two exons, is not able to bind heparin [1-4, 8]. The other splice forms have fragments encoded by exons 6 and/or 7 in many combinations, and therefore have the ability to bind heparin [1-4, 6]. The strength of this binding depends on the amount of amino acid

residues encoded by exons 6 and 7 [1, 2, 4]. The loss of ability to bind heparin leads to a loss of mitogenic activity towards endothelial cells [1, 2]. Thus, it seems that a better understanding of the process of alternative splicing of VEGF-A mRNA, and the identification of the predominant splice forms in different myocardial diseases would be of clinical significance, especially considering the increasing use of VEGF-A in gene therapy. The aim of this study was to estimate the post-transcriptional modifications of VEGF-A mRNA and to determine the concentration profiles of VEGF-A isoforms in non-ischemic dilated cardiomyopathy.

MATERIALS AND METHODS

We performed a molecular analysis of 72 cardiac specimens taken from the explanted hearts of three transplant patients at the time of transplantation. The characteristics of the patients whose hearts were analyzed in this study are shown in Tab. 1. The most important difference was the etiology of the dilated cardiomyopathy (DCM). The first heart showed idiopathic cardiomyopathy, while the other two showed post-inflammatory cardiomyopathy. All the diagnoses were confirmed by histopathological analysis. None of the biopsies revealed necrotic or ischemic changes within the cardiomyocytes.

The dilated hearts were explanted during the transplantation procedure and designated for utilization. Immediately after explantation but before utilization, all the cardiac specimens were taken and prepared for the molecular and histological analyses described below.

Tab. 1. Patients' characteristics.

	Patient 1	Patient 2	Patient 3
Age [years]	50	52	52
Sex	male	male	male
BMI [kg/m ²]	23.0	25.0	20.8
DCM	idiopathic	post-inflammatory	post-inflammatory
CAD	-	-	-
Smoking	-	-	+
Diabetes	-	-	-
Hypertension	-	-	-
RV [cm]	3.1	3.4	3.3
LVd/LVs [cm]	8.6/8.1	8.0/6.2	9.0/8.0
LA [cm]	5.1	4.8	4.7
EF [%]	19	22	16
NYHA class	III	III	III/IV
Hemodynamic state	stable	stable	stable
Inotropic agents	-	-	-

BMI – body mass index, DCM – dilated cardiomyopathy, CAD – coronary artery disease, RV – right ventricle, LVd/LVs – diastolic/systolic left ventricle diameter, LA – left atrium, EF – ejection fraction.

The explanted hearts and coronary arteries were rinsed of blood with cold saline upon explantation. Therefore, the results of the study were not falsified with the expression of analyzed transcripts coming from morphotic elements of the blood.

Next, twelve biopsies were taken from each heart, with 3 biopsies per location (from the anterior, lateral and posterior walls of the left ventricle, and from the interventricular septum – IVS). Two of the 3 biopsies per location were cut twice into three cross-sections according to the borders of their layers (endocardium, myocardium, epicardium), and each cross-section was placed in a separate micro tube. Thus, 6 specimens per location and in total 24 specimens per heart were obtained for molecular analyses.

Immediately after preparation, all these samples were deep-frozen in liquid nitrogen at -135°C due to RNA instability. They were stored at -70°C until molecular analysis, which was directly preceded by an additional microscopic evaluation of all the specimens to ensure there was no contamination from another layer.

The remaining biopsies (one per location, 12 in total) consisted of three layers and were designated for histological analysis. Each was placed in a vial with formalin.

The molecular analyses were done in four iterations, chronologically in the following stages:

1. RNA extraction.

RNA was extracted using a Total RNA Prep Plus kit (A&A Biotechnology). All the RNA extracts were purified with an RNeasy Mini Kit (catalogue #74106, Qiagen, Valencia, CA, USA) to eliminate contamination from genomic DNA in the studied samples.

2. Spectrophotometric quantitative assay of the RNA extracts.

Quantitative assays of the RNA extracts were performed spectrophotometrically using Gene Quant (Pharmacia Biotech).

3. cDNA synthesis and amplification performed via the QRT-PCR technique using an ABI PRISM™ 7700 robot.

During the cDNA synthesis, Tth DNA Polymerase was used, allowing the reverse transcription reaction and the next step of QPCR to be performed in the same tube without the need to change the composition of the reaction mixture. The reverse transcription was performed at 60°C for 30 minutes.

cDNA amplification was performed via the QPCR technique using ABI PRISM™ 7700. As mentioned, this was “one-step” reaction, done in the same reaction mixture that was used for the reverse transcription: 50 μl of solution containing TaqMan Buffer A, 3 mM MgCl_2 , 200 μM dNTP, PCR reaction buffer, 0.5 mM MnSO_4 , 1-10 μg total RNA, 5 U Tth DNA Polymerase and 0.3 μM of each primer, and 0.2 μM of probes labeled with 5' end FAM (6-carboxyfluorescein) and 3' end TAMRA (6-carboxytetramethylorodamin). The thermal conditions of amplification for TaqMan QPCR were: initial denaturation

at 95°C for 5 minutes; 40 two-step cycles of denaturation at 95°C for 30 sec and annealing at 60°C for 1 minute; and a final extension at 72°C for 10 minutes.

The primers and probes, for which the nucleotide sequence was designed with Primer Express™ Version 1.0 ABI PRISM, were used to detect VEGF-A mRNA. All the primers and probes for mRNA amplification were designed to react at the same temperature of melting, and the QRT-PCR was performed in separate tubes simultaneously for all the studied isoforms, the endogenous control, and the templates [9, 10]. The detection of particular isoforms of VEGF-A mRNA was performed using fVEGF primer, rVEGF primers and the sVEGF probe, the nucleotide sequences of which are presented in Tab. 2. The quantitative assessment of the RNA extract was performed based on the number of β -actin mRNA copies, which was also estimated using the QRT-PCR technique with the appropriate primers and probe.

Tab. 2. The nucleotide sequences of particular primers and probes used in QRT-PCR.

Name	primer/probe	Nucleotide sequence
sVEGF*	Probe (for all VEGF-A splice forms)	5'-ACCCATGGCAGAAGGAG GAGGGCAGAATCATCAC-3'
fVEGF	forward primer (for all VEGF-A splice forms)	5'-GCTGCTCTACCTCCACCA TGCCAAGT-3'
rVEGF121	reverse primer for VEGF121	5'-CTCGGCTTGTCACATTTT TCTTGTCTTGC-3'
rVEGF145	reverse primer for VEGF145	5'-GCCTCGGCTTGTCACATA CGCTCC-3'
rVEGF165	reverse primer for VEGF165	5'-GGCCACAGGGATTTTCT TGTCTTGC-3'
rVEGF183	reverse primer for VEGF183	5'-CCACAGGGACGGGATTTC TTGCG-3'
rVEGF189	reverse primer for VEGF189	5'-GCCACAGGGAACGCTCC AGG-3'
rVEGF206	reverse primer for VEGF206	5'-GCTCCAGGGCATTAGACA GCAGCG-3'
P β F	forward primer for β -actin	5'-TCACCCACACTGTGCCCA TCTACGA-3'
P β R	reverse primer for β -actin	5'-CAGCGGAACCGCTCATTG CCAATGG-3'
S β *	probe for β -actin	5'-ATGCCCTCCCCATGCCA TCCTGCGT-3'

*probes labeled with: 5' end FAM and 3' end TAMRA

Simultaneously for all the specimens, and using the same reaction mixture as above, but in separate tubes, a commercially available fragment of the β -actin gene in the dilution series (TaqMan[®] DNA Template Reagents, catalogue #401970, Applied Biosystems, Foster City, CA, USA) was amplified at five ready-made concentrations (1×10^3 , 2×10^3 , 5×10^3 , 1×10^4 , and 2×10^4 copies of β -actin) and in three repetitions for each dilution. The results obtained during the amplification of this quantitative commercial β -actin template were used to draw the standard curve, which was characteristic for the particular analysis. Based on this, the ABI PRISM[™] 7700 sequence detector counted copies of cDNA for the appropriate genes automatically in the studied samples. There were no separate standard curves for particular VEGF-A splice forms. The standard curve for β -actin was used for all the specimens as a secondary template to account for the number of mRNA copies for each sample. Thus, the results for all the VEGF-A splice forms were influenced by the same error. This enabled us to estimate the changes in the expression profiles within particular specimens and myocardial layers, achieving the objective of this study.

The negative control in the QRT-PCR was the reaction mixture with water instead of the template. The other components of the negative control were exactly the same as in the reaction mixture for the studied samples.

4. The evaluation of the amplification's specificity via electrophoresis with polyacrylamide gel.

The identification of the amplification products and the evaluation of their specificity was performed using electrophoresis with 6% polyacrylamide gel. Then, the separated fragments of DNA were stained with 0.4% silver nitrate.

RESULTS

The analysis of VEGF-A transcriptional activity was performed for all 72 specimens from the three explanted hearts. The three hearts showed the presence of all the studied isoforms, i.e. VEGF121, -145, -165, -183, -189, and -206. However, many different expression profiles were found for these splice forms, even within particular layers of the same heart. Maps for particular layers of cardiac muscle with the expression profiles of the studied isoforms were prepared to show this phenomenon (Figs 1, 2 and 3).

Each colour on the map represents a given heart, with grey for the first heart, dark grey for the second, and black for the third. The presence of all the isoforms was only noted within the endocardium of the second heart, in specimens coming from its anterior, lateral and posterior walls (Fig. 1). A similar situation was found in the myocardium, but only in the specimens taken from the anterior and lateral walls of the second heart (Fig. 2). The presence of all the studied isoforms was observed within the epicardium exclusively in biopsy samples coming from the lateral wall of the first and second hearts (Fig. 3).

	heart	121	145	165	183	189	206
anterior	3	■	■	■	■		
	2	■	■	■	■	■	■
	1					■	
lateral	3	■	■	■			
	2	■	■	■	■	■	■
	1		■	■		■	■
posterior	3	■	■		■	■	■
	2	■	■	■	■	■	■
	1						■
IVS	3	■		■	■	■	
	2	■	■	■	■	■	
	1	■	■	■			

Fig. 1. A map of the expression of particular VEGF-A isoforms in the endocardium.

	heart	121	145	165	183	189	206
anterior	3	■			■	■	
	2	■	■	■	■	■	■
	1				■		
lateral	3	■	■	■			
	2	■	■	■	■	■	■
	1		■	■	■		
posterior	3			■	■		■
	2		■	■	■	■	■
	1			■			■
IVS	3	■	■	■	■	■	
	2	■	■		■	■	
	1						

Fig. 2. A map of the expression of particular VEGF-A isoforms in the myocardium.

	heart	121	145	165	183	189	206
anterior	3	■	■	■	■		
	2	■	■	■	■	■	
	1						
lateral	3	■	■	■			
	2	■	■	■	■	■	■
	1	■	■	■	■	■	■
posterior	3	■		■	■		
	2	■	■	■	■		
	1			■			
IVS	3	■	■	■	■	■	
	2			■	■	■	■
	1						■

Fig. 3. A map of the expression of particular VEGF-A isoforms in the epicardium.

Quantitative analysis of particular layers of the studied hearts showed significant differences in the amounts of the analyzed VEGF-A splice forms. The amounts of detected mRNA copies of particular isoforms per 1 μg of total RNA were expressed as the natural logarithm. Within the epicardium of the two hearts with post-inflammatory etiology of cardiomyopathy, the medium-length isoforms were the most common and VEGF206 was the least common. The epicardium of the first heart was characterized by similar proportions of all the VEGF splice forms (Fig. 4).

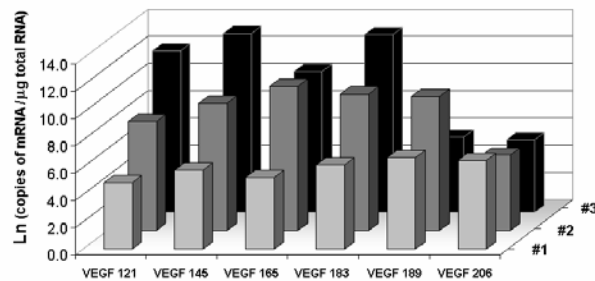


Fig. 4. The expression of particular VEGF-A isoforms within the epicardium of the studied hearts.

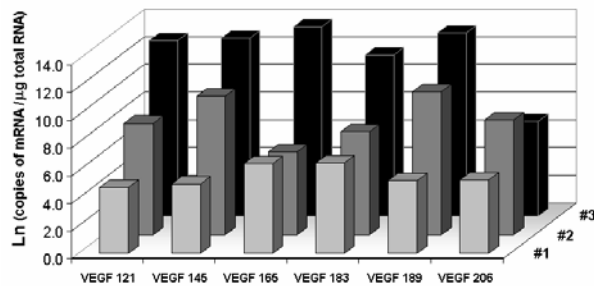


Fig. 5. The expression of particular VEGF-A isoforms within the myocardium of the studied hearts.

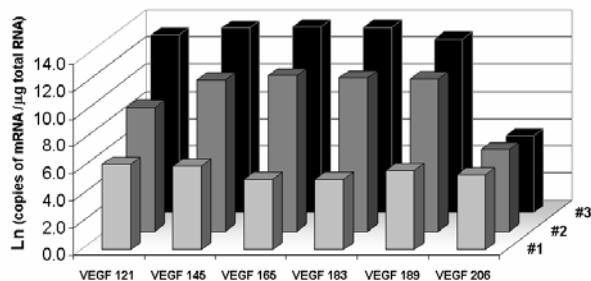


Fig. 6. The expression of particular VEGF-A isoforms within the endocardium of the studied hearts.

Similar proportions of all the VEGF-A splice forms with the exception of VEGF206 were found in the myocardium of the first and third hearts. Significant oscillations in the amounts were observed within the myocardium of the second heart, where the highest levels of VEGF145 and VEGF189 were found (Fig. 5). As in the epicardium, within the endocardium of the second and third hearts, the medium-length isoforms were the most common, and VEGF206 was the least common. The endocardium of the first heart was characterized by similar proportions of all the VEGF-A splice forms. It is also worth noting that the endocardium was the most homogenous layer with respect to the presence and amounts of particular VEGF-A splice forms (Fig. 6).

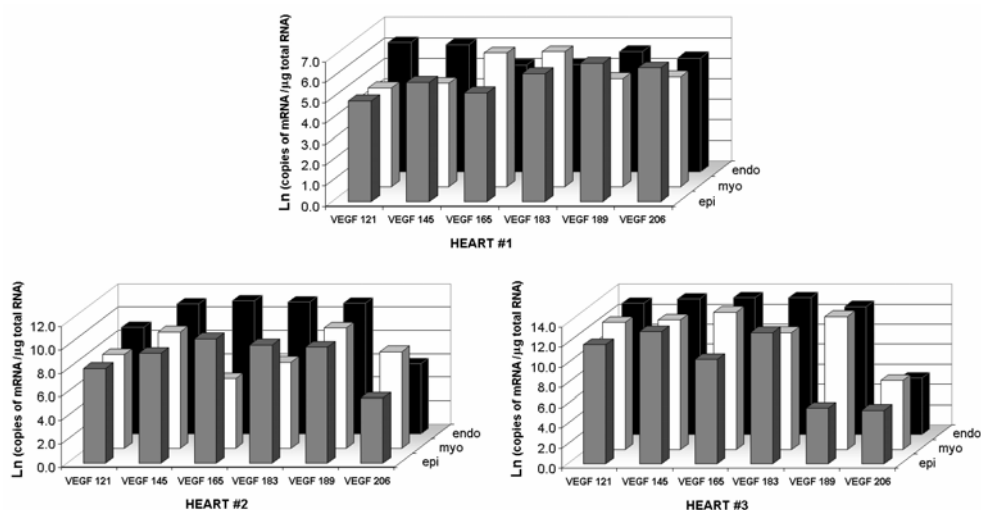


Fig. 7. The expression of VEGF-A isoforms within particular hearts (epi – epicardium, myo – myocardium, endo – endocardium).

The studied hearts were characterised not only by important differences in post-transcriptional modifications of VEGF-A mRNA, the manifestation of which was the presence of many isoforms, but also by significant differences in the amounts of these transcripts. Generally, all the layers of the two hearts with post-inflammatory etiology of cardiomyopathy had significantly higher levels of the VEGF-A splice forms compared to the first heart, which had idiopathic cardiomyopathy (Figs 4, 5 and 6). The exception was VEGF206, the amounts of which were the lowest in all the layers of the second and third hearts. The mRNA levels of VEGF206 in these hearts were similar for the endocardium and even lower for the epicardium relative to the first heart (Figs 4, 5 and 6). Significant differences in the amounts of the VEGF-A splice forms were also observed between particular layers of the same heart (Fig. 7). It was especially strongly expressed in the hearts with post-inflammatory etiology of

cardiomyopathy. The endocardium of the second and third hearts was characterized by the highest expression of all the VEGF-A isoforms, except VEGF206. The first heart was the most homogenous within particular layers and the differences between locations were not as significant as in the post-inflammatory dilated hearts (Fig. 7).

DISCUSSION

There is growing interest in growth factors, and VEGF-A is one of the most popular. The evidence of this popularity is the ever-increasing number of studies on VEGF-A. Despite the numerous studies, there is still a lack of reports on the expression profiles of VEGF-A isoforms in the human dilated heart, especially relative to particular myocardial layers. However, there are some studies where the expression of VEGF was estimated in different heart diseases, especially in ischemia, but that estimation referred to the final product of conversion, which is VEGF protein, usually measured in the serum [11-18]. This method has some faults and limitations. First of all, the estimation of protein concentrations in the serum has a low specificity, especially in chronic and advanced cardiovascular diseases, where chronic hypoxia of the organs and tissues may have a significant influence on the total VEGF concentration in the blood. It is well known that hypoxia is one of the strongest factors that upregulates VEGF-A expression [14, 19-23].

In the acute phase of the disease, the expression of genes and receptors may change profoundly with different strengths and at different times. A consequence of this may be considerable oscillations in the VEGF-A concentrations.

One of the aims of this study was to estimate the expression of the gene encoding VEGF-A in different pathological processes which lead to heart failure. The above-mentioned analysis was performed on the particular layers and with reference to the clinical parameters that are routinely estimated before each cardiac transplantation. It is well known that many factors and comorbidities may influence VEGF-A levels, and one of the most important is hypoxia. The clinical characteristics of the recipients were fortunately quite similar. Thanks to that, the potential baseline bias, which could have influenced the results, was limited. All the patients were male, aged 50-52, and had a similar body mass index, which is especially important in chronic heart failure. None of the patients had coronary artery disease (as also confirmed by histological analysis), diabetes, hypertension, or other comorbidities which could significantly influence the expression of VEGF-A. The echocardiographic parameters and the NYHA functional class were similar for those patients. Moreover, their hemodynamic state was stable, and none of them was treated with inotropic agents. Taking into account all these clinical parameters and the similar findings for all the patients, the results of this study could be explained mainly by the differences in the etiology of the cardiomyopathy. Those hearts

with post-inflammatory etiology of cardiomyopathy had similar divergences compared to idiopathic etiology.

It must also be remembered that the proangiogenic activity of VEGF-A may be influenced by the amount and type of isoforms, which are determined by alternative splicing of mRNA [1, 2, 4]. The mechanisms of that process are still unknown, but most probably the presence of particular VEGF-A isoforms is determined and regulated by metabolic processes and the microenvironment [1, 20, 24-27]. This hypothesis is supported by the results of the presented study, where a significant increase in VEGF-A mRNA expression was observed in hearts with post-inflammatory etiology of cardiomyopathy compared to idiopathic. This also suggests that the profiles of expression of VEGF-A isoforms will change according to the place of biopsy. So far, in the literature, there is a lack of such studies with human myocardial muscle [28]. To the best of our knowledge, this is the first report in the literature on differences in the expression of VEGF-A isoforms with respect to myocardial layers and the location of cardiac biopsy. Lee *et al.*, studying ventricular specimens from patients undergoing coronary bypass surgery, found VEGF protein only in the endothelium that lined the small vessels in the myocardium, unlike hypoxia-inducible factor 1 (HIF-1), which was expressed in both endothelial cells and cardiomyocytes [14]. It seems that the intensity of this occurrence may have prognostic value for patients. Furthermore, in the increasing number of studies in the field of VEGF gene therapy, finding types of alternative splicing of VEGF mRNA and the associated concentration profiles of particular splice forms and receptors may have very important therapeutic implications [29-38].

This study should partially help in better understanding the mechanisms leading to heart failure by finding that the expression of VEGF-A isoforms may vary significantly with respect to myocardial pathology. The idiopathic DCM was characterized by the downregulation of this expression when compared to post-inflammatory DCM. The differences in the distribution and amounts of VEGF-A splice forms observed in this kind of pathology were less expressed within particular layers. On the contrary, the post-inflammatory dilated hearts were much more heterogeneous. However, repetitive and comparable results for samples with post-inflammatory etiology, which revealed considerably higher amounts of VEGF-A splice forms compared to those with idiopathic etiology, give rise to the suspicion that a significant upregulation in VEGF-A mRNA expression is the consequence of myocarditis, and this phenomenon is independent of the biopsy location. If all this data could be confirmed by results obtained from a higher number of hearts and in different laboratories, it could help in working out new methods for diagnosing and monitoring the disease, as with the potential usefulness of the endomyocardial biopsy in molecular diagnosis based on VEGF-A and other proangiogenic factors.

The molecular analysis of the studied myocardial specimens revealed the presence of all the analyzed isoforms of VEGF-A in the hearts, including those which were perceived as characteristic for other tissues, for example VEGF206

[1, 39]. Thus, the studied hearts are characterised by the presence of all the recognised types of alternative splicing of VEGF-A mRNA. The consequence of this is the presence of many different expression profiles of VEGF-A isoforms, even within particular layers of the same heart. For a better understanding of this phenomenon, a map was prepared with the expression profiles of the studied splice forms for particular myocardial layers.

The considerable differences in the qualitative and quantitative distributions of VEGF-A isoforms, even within the same heart, indicate strong transcriptional heterogeneity of studied hearts. As yet unpublished data from our studies on rats also revealed such a strong transcriptional heterogeneity in rat hearts, contrary to, for example, their kidneys. These studies revealed no differences in the levels of VEGF-A and Flt-1 between the studied samples of kidneys [40]. Such homogeneity of VEGF-A mRNA expression, as noticed for the kidneys, was not observed for the rat hearts. Many expression profiles with considerable differences in the amounts of transcripts were established among these rat hearts with normal function. However, this pack of rats was characterized by a lack of the left descending coronary artery, and it cannot be excluded that cellular and functional changes in their hearts were preceded by those molecular divergences (unpublished data). These results support our findings on the strong transcriptional heterogeneity of the studied hearts.

The quantitative and qualitative distribution of particular VEGF-A isoforms was the most similar in the endocardium of the second and third hearts – those which had post-inflammatory etiology and were markedly different from the first heart. It was not the only similarity between these two hearts. As mentioned above, the expression of VEGF-A splice forms was generally significantly greater in the second and third hearts than in the first. This was probably related to the inflammation which had occurred in these similar hearts and with inflammatory mediators released during this process. Many studies proved that different mediators and growth factors regulate VEGF synthesis and even take part in different levels of the angiogenic cascade via VEGF and its receptors [1, 12, 20, 24, 25, 27, 41-43]. The higher transcriptional activity of VEGF-A and the associated high concentrations of VEGF-A isoforms in myocarditis result in bigger cardiomyocytes oedema, because VEGF is a known vascular permeability factor [1-5, 26]. Similarly, upregulation of VEGF-A₁₆₅ and its receptor Flt-1 in the human hearts was reported by Abraham *et al.* due to another strong proangiogenic factor, hypoxia, contrary to its downregulation in dilated but not ischemic cardiomyopathy [23]. De Boer *et al.* also showed decreased expression of VEGF isoforms in patients with idiopathic cardiomyopathy [44]. The results of these two studies [23, 44] concur and suggest that one of the mechanisms responsible for idiopathic DCM is associated with downregulation of VEGF expression at the mRNA and protein levels, contrary to other types of cardiomyopathy, where the microenvironment and metabolic processes enhance proangiogenic activity. The results of this study support this hypothesis, although they have rather the character of a brief communication, because the

analyzed biopsy specimens come from only three hearts. Therefore, we could not draw valid conclusions from this data, and have only summarized it.

In summary we found that:

1. All the studied VEGF-A isoforms were present in the human hearts, including those thusfar considered characteristic for other tissues.
2. Significant differences in the expression of the VEGF-A splice forms with respect to the myocardial layers and the location of the cardiac biopsy were observed in the analyzed hearts.
3. Repetitive and comparable results for samples with post-inflammatory etiology were obtained, and they revealed considerably higher amounts of the VEGF-A isoforms compared to specimens with idiopathic etiology.

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