

The Role of Inherited Vasa Vasorum Remodeling in QBS Microcirculatory Theory of Atherosclerosis

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Abstract

Objectives:

To interpret clinical data collected at the bedside with the aim to formulate a theory of atherosclerosis based on microcirculatory events.

Methods:

The clinical data are collected by means of the Auscultatory Percussion of the Stomach with the aid of Quantum Biophysical Semeiotics (QBS) method and theory. QBS is an extension of medical semeiotics. It is grounded on a multidisciplinary approach that involves chemistry and biology, genetics and neuroscience, chaos theory and quantum physics. It is based on the method of 'Auscultatory Percussion', through which by means of the common stethoscope, it is possible to listen to the signs that the body gives us when diverse trigger-points are appropriately stimulated. The stimuli are used to induce consistent behavior in precise and well defined biological systems of the human body, thus giving local qualitative and quantitative information on the state of health or disease, even potential, being developed but not yet evident by usual clinical trial, effective or even in chronic phase. The QBS method provides very detailed case studies based, e.g., on the latency time, duration, and intensity of the reflexes, which play a central role in such a diagnostic method.

Results:

The clinical and experimental evidences on the functional and structural microcirculatory events of any arterial wall suggest that there is a vasa vasorum inherited remodeling starting from the first decade of life.

Conclusions:

The process of atherogenesis starts from birth in individuals suffering from Arteriosclerotic Constitution-Dependent Inherited Real Risk of CVD, even when there is generally no blood dyscrasia of any kind, and blood pressure is normal.

Keywords: vasa vasorum, clinical, diagnostic method, microcirculation, atherosclerosis

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1. State of the art

According to current studies [1], cholesterol-rich apoB lipoproteins, triglyceride-rich particles (TRPs), hyper-insulinemia and insulin resistance, hypertension, homocysteine, smooth muscle cells and blood-cells, including monocytes and platelets, play a central role in the pathogenesis of atherosclerosis. In particular, a fundamental role is played by cholesterol-rich lipid and protein infiltration of arterial wall, as well as by mechanic action of blood-flow on such a wall, i.e., *shear-stress*, *shear rate* and *stretch stress*.

There is an awful number of pathogenetic theories, that emphasise - from time to time - one of these multiple pathogenetic factors of Atherosclerosis, beginning with Virchow's (1856), Anitchkov's and Chalатов's (1913) theories. Virchow's inflammation theory of atherosclerosis represents a great contribution to the concept of artery lipid retention and thrombosis process. But even Virchow did not expressly stress the concept of atherosclerosis as an autonomic non-inflammatory entity; he called the condition chronic "endoarteritis deformans" [2].

The most recent theories, for instance, the well-known "*Response to Insult Theory*" [3], and "*Response to Retention Theory*" [1], accord that the initial phenomenon is represented by structural and functional endothelial lesion, followed by LDL and other lipid-rich substances retention in arterial wall, but these studies cannot explain the real nature of ATS beginning, nor the extremely location of initial endothelial lesion and the different evolution in diverse arteries of the same patient, as well as among arteriosclerotic subjects.

In fact, contrary to the first hypothesis "Response to Insult Theory" was based on, authors nowadays agree with ATS beginning brought about by functional endothelial lesion, rather than focal endothel loss, with intimal cell deprivation, and subsequent platelet and monocyte adhesion.

Really, the early event in atherogenesis is considered now the functional impairment, i.e., dysfunction of endothels, caused by an awful number of pathogen agents, despite the difficult understanding of local lesion, the so-called minimal lesions [4, 5].

Accordingly, functional endothelial impairment brings about over-production of VCAM1 and ICAM1, i.e., adhesion molecules of cell surface, as well as the increased secretion of biologically active substances (cythochines, growth factors, free radicals, etc.), the reduced production of radical NO, causing monocyte, platelet, and leucocyte adhesion to endothels and derangement of endothelial hemostatic balance, the capillary permeability of plasma proteins and lipids and the arterial tone control [6].

Herman Boerhaave was the first scientist to mention the pathogenesis of ATS, interpreted as a failure of the vasa vasorum to feed the arterial wall, with rudimentary tools for the study of conjunctival microcirculation [7].

The *vasa vasorum*, which are end-arteries, are necessary to maintain normal vessel wall homeostasis. As a matter of facts, inadequate perfusion of the related vessel wall, as deficiencies of these vasa, has been shown to lead to intimal hyperplasia [8]. More recently, the initial hypothesis relating atherosclerosis to increased development of the vasa vasorum was made by Barger et al. in 1984 [9]. Since then, it has been shown that coronary vasa neovascularization takes place early after induction of experimental hypercholesterolemia, suggesting a role for neovascularization in atherogenesis [10]. This neovascularization has been shown to favour second order vasa [11].

Apolipoprotein E-deficient mice given the angiogenic factor vascular endothelial growth factor (VEGF), which leads to increased angiogenesis, it shows a subsequent increase in plaque area while mice administered the anti-angiogenic factors endostatin and TNP-470 show decreased intimal hyperplasia [12]. A decrease in intimal hyperplasia was also observed in a murine study

following chronic endothelin receptor antagonism, which decreases VEGF expression and decreases vasa neovascularisation [13].

These studies provide evidences that neovascularisation of the arterial wall (i.e., impairment of vasa vasorum) is a crucial part of the atherosclerotic process. Abnormalities of the vasa vasorum have also been implicated in the development of neointimal hyperplasia after balloon angioplasty and stenting [14].

In two animal models, local injury to the vascular wall stimulated intimal hyperplasia and adventitial neovascularisation, that was increased by VEGF and PR39 and tempered by the inhibition of VEGF and fibroblast growth factor, leading Khurana et al. to hypothesize recently that intimal hyperplasia has both angiogenesis-dependent and -independent phases [15]. Indeed, it has previously been shown that following angioplasty injury, the number and the density of adventitial microvessels increase in the initial three post-procedural days, then regress.

Kwon et al. evaluated the spatial pattern of neovascularisation, showing that although number and diameter of the vasa, increased after injury, the total vascular area was lower in injured vessels than in control vessels [14].

Deployment of an intravascular stent leads to arterial wall compression and increased resistance within the vasa vasorum, resulting in vascular wall ischemia and subsequent neo-intimal proliferation [16]. Proliferation studies have shown that a tyrosine kinase inhibitor inhibited both neovascularisation and neo-intimal proliferation after coronary stenting, and that the neo-intimal proliferation was proportional to the number of adventitial microvessels present [17].

With the established importance of the coronary vasa vasorum on neo-intimal proliferation in atherosclerosis, angioplasty injury, and arterial stenting, accurate quantification of vasa vasorum number and volume (*i.e.*, *parietal tissue O₂*, *pH*, and *so on*) is a fruitful area of research [18].

The traditional method of vasa vasorum quantification uses histology, but this approach requires staining for vasa vasora endothelial cells and suffers from difficulties such as cutting through metallic stents, inaccuracies due to unperfused vasa, and incorrect data due to a limited number of measured cross-sections. An *in vivo* human method is not plausible, as the coronary microcirculation begins at the level of arterioles of 50µm in diameter and progressively branches into capillaries, 5µm in diameter; these blood vessels are too small to visualize using currently available methods. Three-dimensional microscopic computed tomography (micro-CT) has emerged as an accurate and accessible method [19].

Over the last decades, B-mode ultrasonography at high resolution proved to be a reliable and valid method in recognizing initial arteriosclerotic abnormalities in arterial walls. Intimal and media thickening of the carotid artery has been observed in individuals with risk factors for cardiovascular disorders, proving to be a remarkable sign of the presence of coronary arteriosclerosis as well as of its complications [20].

Despite all these researches, there are still many open questions, such as the well-localized origin of endothelial dysfunction and the relationship between genetic causes and risk factors in atherogenesis. Notably, not all patients with intense hyper-dyslipidemia are atherosclerotic, and there are individuals suffering of IMA, in spite of absent dyslipidemia.

On the other hand, one wonders why a large number of patients with hyperinsulinemia-insulin-resistance, hypertension, hyper dyslipidemia live to old age without getting CVD.

On the contrary, even without risk factors for ATS, in several patients we can see the onset of IMA and stroke. Notoriously, CVD occurs even in individuals without risk factors - about 300 - while the CVD may be absent in smoking subjects with impaired blood counts and hypertension.

The pathogenesis of atherosclerosis in the first decade of life and the reasons why the ATS arises in well-localized area and evolves differently, both in the single patient and from patient to patient, are unknown and not yet clinically explored.

There is not a satisfactory explanation to the fact that arteriosclerosis is initially localized only in very limited points of the arterial wall, i.e., if ApoB lipoproteins rich in cholesterol play a very central role in atherogenesis, then it is not clear why these particular lipoproteins are not seen everywhere, that is, in all parts of arterial walls, but only in very limited areas.

It is unclear the reason why particles rich in triglycerides - very abundant in the blood of each individual in the post-prandial period - penetrate only in a well-defined point of a wide arterial wall, saving most of the wall itself.

Furthermore, the association between decreased reactivity of brachial artery and intimal-media thickening of carotid artery as markers of early arteriosclerosis has not yet been explored from a clinical point of view.

Finally, according with recent studies [1, 21], an enzyme, acid sphingomyelinase¹, secreted by endothelial cells, plays a major role in the penetration and retention in the arterial wall of atherogenic molecules such as TRP, particles are rich in triglycerides, the small very low-density lipoproteins (sVLDL), the intermediate-density lipoproteins (IDL). It is unknown if a clinical and pre-clinical diagnosis can corroborate this point of view.

For all these reasons, in the next chapters we explore an original clinical approach for vasa vasorum and microcirculatory bedside evaluation with the aid of Quantum Biophysical Semeiotics (QBS) theory and method which will allow us to propose a new unified theory of atherosclerosis which take in account all the previous fragmented approaches as a whole.

2. The Inherited Real Risk of Atherosclerosis

The state of the art described in the previous chapter refers to patients with either pre-metabolic or metabolic syndrome, and involved by atherosclerosis, even if in their early clinical stages, i.e., the very early *functional-structural* disorder of macrovascular wall.

In the present article, we propose that a subject clinically health could be at heritable risk of atherosclerosis (Inherited Real Risk of Atherosclerosis), i.e., pre-metabolic² syndrome [22], or with atherogenesis in its different pre-clinical stages [23], although silent, asymptomatic, even if usual clinical tests are normal and they do not reveal any abnormality.

In accord with the studies of one of the authors, this is due to genetic alteration of mit-DNA evidenced by the “Congenital Acidotic Enzyme-Metabolic Histangiopathy” (CAEMH), a unique mitochondrial cytopathy that is present at birth and open a new, original way to medical diagnosis and therapy [24].

This is possible with the aid of “Quantum Biophysics Semeiotics” (QBS) [25], a new discipline in the medical field and an extension of the classical semeiotics with a scientific trans-disciplinary approach, i.e., with the support of quantum and complexity theories.

CAEMH is the *conditio sine qua non* of inherited degenerative pathologies such as cancer, T2DM, cerebral disorders and cardiovascular disease (CVD). The presence of intense CAEMH in a well-defined area (i.e., myocardium) is due to gene mutations in both n-DNA and mit-DNA [25]. This is the basis for one or more QBS constitutions³ [27], e.g., atherosclerotic QBS constitution⁴, which could bring about their respective Inherited Real Risks⁵ (IRR), i.e., IRR of CVD characterized by microcirculatory remodeling, intense under environmental risk factors⁶.

In accord with Angiobiopathy theory [28] microvessels, related parenchyma and genome (i.e., mit-DNA and n-DNA) are intimately related, so that the study of microvascular oscillations can give us valuable information on related parenchyma's patho-physiology.

CAEMH modifies mit-DNA of parenchyma as well as vessel wall cells, including those of vasa vasorum. Parenchymal alterations parallel the cell alterations of vessel wall. Parenchymal cells need of less blood supply than the normal one, bringing about vasa vasorum microcirculatory remodeling. In this case, we can clinically observe, from a functional QBS point of view, an

impairment between vasomotility⁷ and vasomotion of the related microvessels' oscillations, which reflects structural new-born abnormalities represented by type I, subtype b) aspecific, new-born pathological Endoarteriolar Blocking Devices⁸ (EBDs) [29]. As a consequence, arteriovenous anastomoses (AVA) are persistently open and there is a centralization of blood-flow, which causes tissue acidosis, thus SMC proliferation (polyamines) and migration (FMFs).

Furthermore, the 'Inherited Real Risk' (IRR) of CVD is associated to endothelial dysfunction⁹, bed-side recognizable in an easy and reliable way, at rest as well as under stress tests [30]. As a consequence of the above, briefly referred remarks, according with QBS theory, physicians can observe the presence of typical newborn pathological EBDs in microvessels [31], which play a central role in the IRR of CVD. In addition, pathological EBDs bring about a turbulent flow-motion and lowered shear stress, causing local blood-sludge, as reduced Oxygen Recovery Time demonstrates [25]. Local endothels are similarly altered (HP regions) due to embryological reasons [32]. As a consequence, cell infiltration, lipid and protein retention may occur later at this level, contributing to media-intimal thickening.

With the aid of QBS theory and method, doctors can bedside assess microvascular dynamics [33]. The microvessels carry on a motor activity, autochthonous and chaotic deterministic [34], which represents one of the most remarkable manifestations of microcirculatory hemodynamics, characterized by a flow-motion and rhythmically fluctuating hematocrit due to the particular nonlinear behaviour [35] of both vasomotility and vasomotion.

Microcirculation shows three basic types of activation, a part from many transitional forms (table 2, sixth column):

- 1) type I, Associated (the term 'associated' means that vasomotility and vasomotion show the same physiological behavior);
- 2) type II Intermediate, partially dissociated (pre-metabolic syndrome, dissociated because vasomotility and vasomotion have a different behavior: briefly, vasomotility is increased to maintain normal vasomotion);
- 3) type III Completely Dissociated (pathological microcirculation, typical of overt disease).

In case of 'Inherited Real Risk' of CVD, there is a functional alteration of localized vasa vasorum dynamics evidenced by an impairment of vasomotility and vasomotion (microcirculatory activation, type 2, dissociated) as well as structural abnormalities such as the presence of pathological EBDs [23, 26, 29, 31]. These functional and structural abnormalities increase and worsen along time, with the evolution of the IRR of CVD (pre-clinical stage) to the overt pathology (microcirculatory activation, type 3, dissociated), revealing an Allegra's syndrome [36].

Interestingly, internal, medial and external vasa vasorum are more abundant in the veins, although thin, than in related arteries, with the unique exception in pulmonary vessels, according to QBS experimental evidences (data not yet published). In fact, because lung artery carries venous blood, no large amount of vasa vasorum needs to wall nutriment. As a matter of facts, the less oxygen in the vein lumen accounts for the reason veins are provided by a more quantity of vasa vasorum.

Through the QBS method illustrated in the next chapter, there are experimental evidences, by means of a QBS diagnosis in 120 subjects with IRR of CVD, which allow us to define the pathogenesis of atherosclerosis in the first decade of life, in subjects with atherosclerotic constitution and IRR of CVD, due to the clinical investigation of the arterial wall in vivo, when the composition of the blood is generally still normal [37].

In these subjects, from the birth till the end of first year of life (table 1), we can diagnose vasa vasorum with IRR of CVD, a slight dissociated microcirculatory activation type II, worsening during efforts and Allegra's Syndrome, while pH shows at rest normal physiological values (first pre-clinical stage of ATS). Later one, till the fifth year of life, the type II dissociated microcirculatory

activation worsens, even at rest, pH decreases, while poly-diamines, Fibroblast Stimulating Factor and hyaluronic acid fragments significantly increase. There are cells, e.g., smooth muscle cells, whose proliferation and migration stimulation bring about artery intimal thickening (second pre-clinical stage of ATS).

From the age of five till the age of ten, in the above mentioned subjects, there is type III dissociated microcirculatory activation, the pH continues to decrease, and thus damages worsen in local endothels and tissue. All these facts bring about cellular infiltrations, initial plaques' formation and subsequent artery lumen narrowing, but exclusively where intense CAEMH is present (third pre-clinical stage of ATS).

Table 1

The clinical and experimental evidences provided by QBS diagnosis in 120 subjects with IRR of atherosclerosis induce us to propose a new unified theory of atherosclerosis based on arterial wall microcirculatory bed behavior.

From the birth, QBS method allows to clinically recognize, at the bed-side, microcirculatory modifications, by the evaluation of abnormalities of haemoderivative structures (i.e., AVA, including EBDs, from the functional view-point) and the reduced arterial dilation, always associated to intimal-media thickening, based on endothelial insufficiency, which seems to play a primary role in the most important CAEMH-dependent alterations of vasa vasorum.

To summarize, the proposed unified microcirculatory theory of arteriosclerosis is as follows: heritable endothelial impairment, caused by intense CAEMH and worsened by a lot of environmental risk factors (about 300), only partially known, brings about lowering synthesis of NO radicals, increased secretion of vasoconstrictors substances, so that the endothelial-dependent haemostatic unbalance can predispose, in the examined individuals, to blood mononuclear cells, e.g., monocytes, NT-cell, and platelets, adhesion, and medial smooth muscle cells proliferation. Their migration to the intima, monocytes-derived macrophages, as well as lipoproteins storage in the arterial wall, will be observed with the progression of the pre-metabolic syndrome towards the metabolic one.

The genetic component represented by intense CAEMH in well-defined arteries, according to the paramount phenomenon, known as mitochondrial heteroplasmy and the IRR of CVD, causing artery wall acidosis, are the essential bases of atherogenesis. They explain the onset of CVD in individuals without significant environmental risk factors [38], the exact location and nature of the HP and LP zones, the seat of limited Minimal Lesions, the disease course completely different in the same individual, from artery to artery, and among patients with ATS, etc.

As briefly mentioned above, we highlight three key points at the base of our 'Microcirculatory Theory of Atherosclerosis', that give satisfactory answers to till now un-resolved problems, concerning the physio-pathology of atherogenesis:

- 1) the presence of a mitochondrial cytopathy, mainly functional, termed CAEMH;
- 2) the phenomenon of mitochondrial heteroplasmy¹⁰, intra and extra-cellular;
- 3) The well-circumscribed acidosis in the arterial wall, initiating in the second year of life, caused by the remodeling of the vasa vasorum, and the subsequent permanent hyperstomy of AVA, dependent of genome alteration of local parenchymal cells, most affected by CAEMH.

The clinical QBS diagnosis in the first decade of life, starting from the birth, and the analysis of the collected information provide a compelling explanation for the onset of vessel-wall acidosis, caused by the remodeling of the vasa vasorum. It explains thus the phenomenon and important role of mitochondrial heteroplasmy and the circumscribed endothelial dysfunction (HP Zone endothels), where CAEMH is more intense, the altered synthesis of GAG by the local cells

(fibroblasts, smooth muscle type secreting cells, endothelial cells, etc.), interested in the intense impairment of mit-DNA and n-DNA, and in particular the impaired synthesis of hyaluronic acid in its three different forms, as provided by the clinical QBS assessment of the glycocalyx from the second year of life [39, 40].

On the basis of these data a clinical satisfactory explanation of the penetration - in pathological quantities - in the sub-endothelial space of molecules rich in lipids is possible, even in the absence of significant dyslipidemia - almost never present in these initial stages - and their retention, followed by immobilization in the matrix of the intima by a physiological acid sphingomyelinase, that only in such an environment, by reduced pH, can carry out its activities in an optimal manner. The isoform of acid sphingomyelinase is more active in acid environment¹¹, as the pH of the arterial wall where there is IRR of CVD after the first year of life¹², in accord with the 'QBS Microcirculatory Theory of Atherogenesis', here proposed, i.e., from the second year of life, then in the second stage of our classification of the atherogenic events. During this period, there is generally no increased lipidemia because sVLDL, ILDL, LDL and TrPs are still in normal ranges.

It follows that the events described by some authors [1] happen later than the first decade of life and they cannot be considered as the initial process atherogenesis; moreover, in accord with their theory, they should happen in all the arterial walls of all people [41].

The numerous proteolytic and lipolytic enzymes of arterial intima, capable of modifying lipoproteins and responsible for their retention in the arterial wall, are particularly represented where there are atherosclerotic changes, hence later than the first decade of life.

Furthermore, also other enzymes, sphingomyelinase, SMase, and phospholipase A2 (PLA2), although physiologically present, hold lipoproteins rich in cholesterol and triglycerides in the wall, only in atherosclerotic subjects¹³.

Mast cells provide chymase and trypsin, macrophages and smooth muscle cells secrete metalloproteinases and cathepsin [42]. However, these cells producing enzymes that modify and retain the arterial wall agglomerations of various lipoproteins are not present in the intima in the first year of life of the individual affected by IRR of CVD, i.e., when the vessel wall pH is not decreased at rest, while it lowers in the movements of a restless baby, exactly for the reasons above mentioned.

The proteoglycans of the arterial wall, represented mainly by the hyaluronic acid in the three structurally and functionally different forms at the end of three different synthetic routes, show an altered relationship only in the sites where there is IRR of CVD, but starting from the second year of age, as clinically diagnosed by glycocalyx evaluation (see table 2, column 4th). The ability of modified GAG to bind in excess cholesterol and triglyceride-rich molecules (aggregated especially under the influence of the previous ASMasi), preventing them from leaving the arterial wall, subsequently emerges, later than the first vessel-wall abnormalities observed by QBS diagnosis.

At this point, we emphasize the important role plays in microvessel dynamics by the physiological viscosity of Interstitial Fundamental Matrix, which appears altered from the second year of life in subjects predisposed to ATS. As a matter of fact, free and bound water relation is clearly modified by hyaluronic acid alterations.

3. QBS method & diagnosis

The QBS method allows the early diagnosis of the most severe diseases, e.g., solid and liquid forms of cancer [43], Type II Diabetes Mellitus [44], coronary heart diseases [45, 46], lithiasis [47], even at birth, in their potential, very initial stage, as the IRR of CVD [23, 37].

Doctors can evaluate with a stethoscope, by performing auscultatory percussion of any viscera (i.e., stomach, ureter), mitochondria functions, as well as the behavior of any biological system [48, 49]. The presence of the IRR of many diseases, linked with one or more 'QBS

Constitutions' (i.e., atherosclerotic 'QBS Constitution' which can bring about the IRR of CVD), can be clinically diagnosed from birth so that a personalized prevention strategy can be realized only on those at real risk (with IRR of any disease) [29].

The objective QBS examination allows physician to bedside recognize and quantify, in a few minutes, the presence of 'Inherited Real Risk' (IRR) of atherosclerosis or overt ATS, even initial, the precise site, its severity and its evolution monitored over time, through the evaluation of several semeiotics signs, i.e., assessing vasomotility, vasomotion, AVA and typical pathological EBDs [23, 33, 37, 50, 51].

For example, the arteries' evaluation is done through the auscultatory percussion of ureteres [23, 33, 35]. In particular, the slight, mean-intense and intense digital pressure upon any large artery allow to assess respectively the artery thickening (ureteral reflex in toto) and the vasomotility and vasomotion of vasa vasorum¹⁴ (slight pressure), the artery vasomotion (mean – intense pressure), and the coronary artery compliance - intense pressure under insulin peak test, adiponectine test, boxer's test¹⁵, Restano's and Valsava manoeuvre¹⁶ (table 2, column 7th) [25, 52, 53] -, while the ungueal pressure permits to diagnose the cytochine tissue level. All these data, collected by dynamic tests, are abnormally modified in individuals at IRR of CVD, even in the two first decades of life [23]. They clearly show the very early *functional-structural* disorder of macrovascular wall, however, preceded by EBDs abnormalities of related microvessels, which could be considered the first and essential alteration (table 2, column 5th) [23, 37].

Table 2

These experimental evidences corroborate, from a clinical point of view, those of other authors [54-59], performed with sophisticated methods, because they indicate, as markers of early arteriosclerosis, the association between decreased reactivity of brachial artery and intimal-media thickening of carotid artery, present in young people with family history positive for *premature* myocardial infarct. This association is interesting, because the abnormal vasodilatory response to achethyl-choline (Valsalva's Manoeuvre) and endogenous insulin can be easily evaluated at the bed-side, in individuals earlier involved by microvascular dysfunctions, including AVA, functionally speaking, and EBDs [50].

In following, we briefly resume the easier way for the diagnosis of IRR of CVD: the Gastric Aspecific Reflex (G.A.R.) through the 'Auscultatory Percussion of the Stomach' [49].

In health, without any "IRR of CVD, i.e., in absence of the "variant" Reaven's syndrome [45], the intense digital pressure applied upon the projected skin area of any artery (i.e., carotid artery) does not provoke a simultaneous gastric dilation and the contraction of the antral-pyloric region, i.e., negative semeiotic sign, absence of CVD and of ATS Constitution, termed negative Antognetti's sign [23, 60, 61], hence inducing local metabolic regulation of Tissue Microvascular Unit (T.M.U.), i.e., activating the Microcirculatory Functional Reserve (MFR) [33].

On the contrary, in subjects with ATS Constitution, under the above mentioned experimental condition, the identical manoeuvre brings about a simultaneous G.A.R.; i.e., the stomach dilates (less than 1 cm in case of IRR of CVD, at least 1 cm or more in case of overt ATS). Interestingly, such a reflex is small, less than 1 cm, if there is IRR of disease [23].

As the IRR of CVD is recognized, we should refine the diagnosis in order to determine the severity of the metabolic (ATS in progress) or pre-metabolic (grade of evolution of IRR of CVD) syndrome [61, 63]. The gastric diagnosis is consistent and dually reflects the informative nature and quality of parameters collected by QBS microcirculatory investigations that are in accord with clinical microangiology [33]. The patho-physiology of QBS reflexes is based upon local microvascular conditions [23].

In a supine healthy subject, psycho-physically relaxed, with open eyes, aiming to lower significantly melatonin secretion, a digital pressure of “mean” intensity, applied upon the skin projection’s area of ATS’s trigger points brings about G.A.R., whose latency time (Lt), duration (D), and intensity (table 2) inform on pH, tissue oxygenation - at rest, as well as under stress situations - and Microcirculatory Functional Reserve (MFR) [52].

Clinical and experimental evidences [23] demonstrate that tissue pH is related to the reduction of latency time (Lt) and to the extension of G.A.R. duration, which parallels the local Microcirculatory Functional Reserve (MFR), calculated easily as the disappearing time of G.A.R. before the appearance of the next one [37].

In health, a digital pressure of “mean” intensity, applied, e.g., on ATS trigger points (skin projection of any large artery, i.e., carotid artery), brings about a G.A.R. after a latency time (Lt) of 10 seconds (table 2, 1st column). G.A.R. lasts less than 4 sec., soon thereafter disappearing for 3-4 seconds. Afterwards, a second reflex occurs. The duration of G.A.R. unfolds the MFR activity of related microvessels, thus correlated with the function and anatomy of the microcirculatory bed, the T.M.U. At this point of investigation, the physician quickly interrupts the digital pressure for exactly 5 seconds. Then, Lt of G.A.R. is evaluated again: Lt raises to 20 seconds, the G.A.R. lasts less than 4 seconds, disappearing after roughly 4 seconds: these values evidence a *physiological preconditioning* (table 2, 2nd column) [50].

In summary, when digital pressure is of “mean” intensity, physiological Lt of the G.A.R. is 10 seconds at the first evaluation (*basal-line value*), but it increases doubling the Lt in the second one, due to the physiological activation of MFR. Importantly, in the same subject, reflex duration is varying from more than 3 sec. and less than 4 sec.

In individuals with IRR of CVD, *base-line* Lt is yet physiological during the first evaluation (10 seconds). However, the G.A.R. lasts 4 seconds or more and it disappears for less than 3 seconds. Moreover, preconditioning results “pathological”, as Lt is less than 20 seconds: these values give evidence of a *pathological preconditioning*. Interestingly, in patients with ATS, even clinically silent, the *basal value* of latency time of the G.A.R. appears to be less than 10 seconds at first evaluation and becomes lower in the second one, in relation to the seriousness of underlying disorder.

In healthy subjects the *preconditioning* brings about, as natural consequence, an optimal tissue supply of material-information-energy, by increasing the local *flow-motion as well as flux-motion*. On the contrary, if the IRR of CVD is present, *preconditioning* data are almost the same as the basal ones, but Lt is a little shorter than physiological one. Finally, in overt disease, *preconditioning* shows an altered and shorter Lt of reflex in relation to the seriousness of the underlying disorders.

At this point, we come back to the former example: in the initial phase of ATS (first decade of life), which evolves very slowly toward successive phases, QBS “basal” data can seem “apparently” normal. However, under careful observation, the duration of the G.A.R. is equal or more than 4 seconds (the normal value, NN, is less than 4 seconds), indicating a local microcirculatory disorder (table 2, column 3rd).

In these cases, *preconditioning* allows in a simple and reliable manner to recognize the pathological modifications, mentioned above, which indicate the altered physiological adaptability, even initial or slight, of the biological system to changed conditions as well as to increased tissue demands. The various QBS parameters, related to a defined biological system, parallel and are consistent with the data of *preconditioning*.

In the pathogenesis of atherosclerosis one more important diagnostic value is played by QBS glycocalyx evaluation. The easiest and reliable way to stimulate glycocalyx is as follows: in health, physician assesses the fluctuations of upper ureteral refolex (vasomotility) with a small intense

digital pressure upon any large artery [39, 40, 64]. At the precise moment, upper ureteral reflex disappears, doctor brings about an “intense” digital pressure on the same *large artery, e.g., femoral artery at groin*. “Simultaneously”, an upper ureteral reflex appears, showing its highest intensity [23]. On the contrary, in presence of glyocalix dysfunction related to atherosclerosis or IRR of CVD in that site [37], indicating an impairment of both nDNA and mit-DNA, under identical above-described experimental condition, the latency time of upper ureteral reflex, is between 2 and 5 seconds or more, in relation to the severity of underlying disorder (table 2, 4th column).

4. QBS pre-primary and primary prevention

QBS tools are not only useful for diagnostic purposes, but also for therapeutic advices, because they are able to measure the microcirculatory activity before and after each preventive therapy’s treatment, in order to understand the effectiveness of remedies.

Some years ago, one of the author [27] let us an open question: “Are QBS Constitutions and Inherited Real Risk of degenerative pathologies reversible?”

Through a proper prevention treatment termed ‘type A’ or ‘green’ therapy a genetic reversibility for future generations is possible [65], but this could not be enough for the current generations, especially under environmental negative conditions.

The green therapy¹⁷ stimulates the activity of mitochondria by acting on metabolism (chemical processes), peptides’ net (electric-electronic processes), but also improving, normalizing mitochondrial and tissue oxygenation, expression of the normal operation of mitochondrial oxidative phosphorylation. Indeed, the mitochondrial functional cytopathy above mentioned (CAEMH) is the *conditio sine qua non* of more frequent and severe human diseases.

By this way tissue oxygenation and mitochondrial activity are improved, mitochondria are running well, but it remains the genetic alteration of mit-DNA: CAEMH, QBS Constitutions and IRR of diseases are still positive, but the IRR of CVD becomes ‘residual’. This means that a continuative ‘type A’ therapy averts the risk that the disease can emerge, despite the genetic problem is not yet healed.

Under a ‘green’ therapy the tissue acidosis is reduced. This kind of therapy must be continuative to maintain a sufficient level of mitochondrial and tissue oxygenation and the risk “residual”, otherwise the Lt would decrease restoring the IRR of CVD. The ‘type A’ therapy cures but it does not heal.

One of the authors has recently discover a new class of treatments for preventive purposes termed ‘type B’ or ‘blue’ therapy¹⁸ [66], in accord with the Principle of fractal Genome Recursive Function (PRGF) according to Andras Pellionisz [67, 68].

Under a ‘blue’ therapy, an extremely high microcirculatory activation is observed. QBS diagnosis ascertains the healing of CAEMH and of IRR of CVD in subjects with ATS Constitution in its early pre-clinical stages, i.e., in the first decade of life or anyway before the onset of the pathology itself. From the beginning of this kind of therapy, an intense DNA’s reprogramming and genome’s restructuring activity starts up and lasts for about 9 months. During this period of genetic restructuring and normalization, QBS monitoring states that the high microcirculatory activity gradually diminishes, but it remains slightly activated till the end of the ninth month, a period of plausible normalization of the genetic restructuring just completed, before ceasing and stabilizing at rest.

After about 9 months from the beginning of treatment, the ‘blue’ therapy’s effects are over, while in case of continuative ‘green’ therapy the microcirculatory activation still persists. The ‘type A’ class of treatments must continue along time to prevent the resurgence of the IRR of CVD and of its pre-clinical degenerative evolutionary process (pre-metabolic syndrome) which could lead, soon or later, to the metabolic syndrome.

Conclusions

The 'QBS Microcirculatory Theory of Atherosclerosis' is based on microcirculatory events observed with the stethoscope in every arterial wall, from the birth and during the first decade of life. It provides original and satisfactory solutions to problems so far open.

From the moment of birth, this theory allows us to recognize and locate, on a very large scale and without any monetary cost, the IRR of CVD (through Antognetti's sign) and the predisposition to arterial calcification.

According to our theory, the process of atherogenesis starts from birth in individuals suffering from Arteriosclerotic Constitution-Dependent Inherited Real Risk of CVD, even when there is generally no blood dyscrasia of any kind, and blood pressure is normal. The first, initial, fundamental stage of the Natural History of the ATS is a mitochondrial cytopathy, termed CAEMH.

The related, fascinating phenomenon of mitochondrial heteroplasmy, inter-and intra-cellular, allows us to understand finally the well-circumscribed location of pre-clinical Minimal Lesions (HP zones) located in some limited areas of the arterial tree, but not in all, explaining the different course of the disease. The subsequent, arterial wall acidosis, brought about by local vasa vasorum remodeling, represents the third paramount etio-pathological factors of atherogenesis according to 'QBS Microcirculatory Theory'.

The altered composition of the interstitial matrix, including the glycocalyx, is fundamental in the first decade of life, when LDL is normal, but there is already artery-wall acidosis, caused by microvascular remodeling of vasa vasorum, characterized by typical, newborn-pathological, type I, subtype b), aspecific, EBDs, causing "permanent hyperstomy" of local AVA, type I, group A and B, and the type II, group A and B, according to Bucciante, lowered shear stress and turbulent flow-motion in arterioles and nutritional capillaries.

These very initial, localized changes of artery wall, evaluated in "quantitative" way with a stethoscope during the first ten years of life, playing a central role in atherogenesis, disappear, if recognized promptly, under the 'blue' therapy. The 'blue' therapy, used in a timely, optimal and personalized manner, cures the IRR of CVD, according to the Principle of Recursive Fractal Genome Function of Andras Pellionisz, clinically corroborated by QBS experimental evidences.

Finally, QBS method makes possible the therapeutic monitoring of atherosclerotic lesions.

<p><u>Stage I, at Birth → 1st Year.</u></p>	<p>Vasa vasorum with Inherited Real Risk of CVD. Type II, “slight”, dissociated Microcirculatory Activation, worsening during efforts, Allegra’s Syndrome: at Rest: pH NN.</p>
<p><u>Stage II, after 1st Year → 5th Year</u></p>	<p>Type II “intense”, dissociated Microcirculatory Activation, even at Rest → pH ↓ → Poly-diamines and Hyaluronic Acid Fragments ↑ → Cell Proliferation-Migration Stimulation → Artery Intimal Thickening.</p>
<p><u>Stage III, after 5th Year → 10th Year</u></p>	<p>Type III dissociated Microcirculatory Activation → pH ↓ → local Endothel and Tissue Damage → cellular Infiltration and initial Plaque Formation → subsequent artery Lumen Narrowing</p>

Table 1. The three stages of atherosclerosis in the first decade of life (explanation in the text).

Artery - Gastric Aspecific Reflex (G. A. R.) mean-intense digital pressure applied upon any large artery (brachial, phemoral artery at groin, carotid artery, a.s.o.)

Latency time (Lt) of G.A.R. in sec.	Latency time of G.A.R. after pre-conditioning (pause of 5 s.)	G.A.R. Duration, i.e., MFR, in seconds	Glycocalyx evaluation (Lt of upper ureteral reflex)*	EBDs	Pre-conditioning	Valsava M. & Boxer test	Diagnosis
Lt = 10	Lt = 20	3 < MFR < 4 normal MFR, associated activation, outcome +	Lt = 0 simultaneous intense upper ureteral reflex appears	Normal EBDs physiological function	Type I, Physiological Tissue Microvascular Unit	Lt = 10	Health
Lt = 10	Lt < 20	MFR = 4 compromised MFR, dissociated activation, outcome ±	Lt = 2 – 3 Glycocalix dysfunction	Normal, slightly modified EBDs function, small number of pathological EBDs	Small Type II Intermediate tissue microvascular unit	Lt = 10	Atherosclerotic Constitution
7 < Lt < 10	14 < Lt < 20	4 < MFR ≤ 5 growing compromised MFR, dissociated activation, outcome ±	Lt = 3 – 4 Glycocalix dysfunction	Modified EBDs function, increasing number of pathological EBD	Worsening Type II Intermediate tissue microvascular unit	Lt < 10	Inherited Real Risk of CVD
Lt ≤ 7	Lt < 14	MFR > 5 absent MFR, dissociated activation, outcome –	Lt ≥ 5 Glycocalix dysfunction	Pathological EBDs function, large number of pathological EBDs	Type III Pathological tissue microvascular unit	Lt < 7	Overt CVD

Table 2. Legend: MFR (Microcirculatory Functional Reserve); EBDs (Endoarteriolar Blocking Devices); Lt (Latency time); CVD (Cardiovascular Disease); G.A.R. (Gastric Aspecific Reflex); Valsava M. (Valsava Manouvre).

**Glycocalix evaluation: Physician assesses the fluctuations of upper ureteral reflex (vasomotility) in any large artery, e.g., phemoral artery at groin. At the precise moment, upper ureteral reflex disappears, doctor brings about an “intense” digital pressure applied any large artery, e.g., phemoral artery at groin, looking at the latency time of this reflex*

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¹ These molecules themselves have lower affinity binding to proteoglycans of the arterial wall of LDL. These authors make use of physiological conditions - enzymes mentioned, including the acid sphingomyelinase note - to justify the crucial role played by GCG (especially if it's modified, but they are considered normal) of amorphous interstitial ground substance in atherogenesis. Note The various isoforms of sphingomyelinase are distinguished by their subcellular localization and for the pH necessary for the enzymatic catalysis more effective. The most interesting are those neutral sphingomyelinase (NSMase-1 and -2) with distribution in various cellular organelles and the acid sphingomyelinase (A.SMase), exclusively localized in lysosomes.

² Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. It is also known as metabolic syndrome X, syndrome X, insulin resistance syndrome, Reaven's syndrome. The pre-metabolic syndrome, as defined by one of the authors, is the syndrome that precedes the metabolic one, and is linked with congenital real risks and their related QBS constitutions.

³ QBS constitutions, detectable since birth, are the inherited congenital ground or terrain of well defined potential diseases clinically hidden, which can last several years before appearing, in the slow transformation process from potential (pre-metabolic syndrome, pre-clinical stages) to effective pathology (metabolic syndrome)

⁴ The existence of this QBS constitution is corroborated by the relation between abnormal reactivity of arterial wall and intimal-media thickening, as shown by high resolution clinical diagnosis.

⁵ Real Risk – RR - means any mutation, limited at level of cells belonging to a well-defined biological system - for example, beta cells of islets of Langerhans, for diabetes - which occurs in one or more cells when ATP decreases strongly for any reason.

⁶ In accordance with QBS the most important risk factors for atherogenesis, i.e., tobacco smoking, dyslipidaemia and arterial hypertension, are innocent bystanders if there is not atherosclerotic constitution and IRR of ATS [references].

⁷ In all tissues, a part from their local different architecture, microvessel diameter oscillates rhythmically during time. The term *vasomotility* refers to small arteries and arterioles sphygmicity, according to Hammersen, and *vasomotion* is the subsequent oscillation of capillaries and post-capillaries venules diameter.

⁸ The Endoarteriolar Blocking Devices (EBDs) are a kind of dam which by opening and closing regulates blood flow in microvessels directed to the parenchyma. If these EBDs are tough, rigid, inelastic, there is a Real Risk of disease. There are EBDs Type I - located in small arteries, according to Hammersen -, and Type II – they can be found in the arterioles that are between small arteries and capillaries -: only type II is ubiquitous, in the sense that it is observed everywhere, in all arteries. Even these physiological types get sick or old. However, the other types, pathological-new-formed, are expressions of the Real Risk of potential disease, they are more occlusive, but through therapy they can be transformed from subtype a) pathological, to subtype b) aspecific, and then to "physiological" type, decreasing gradually their amount. EBDs play a primary role in the regulation of local microcirculatory *flow-motion*: when this is abnormal, there is congenital microvascular remodeling and EBDs bring about impairment of the Microcirculatory Functional Reserve (MFR), which contribute to affect the 'Real Risk' of disorders, like ATS, whose onset shall possibly occur after years or decades.

⁹ There are mitochondria also in endothels, although in small amount. In the lining of the arteries (endothelial cells) and the smooth muscle cells in the walls of the arteries. The endothelial dysfunction is likely to be multi-factorial in these patients and it is conceivable that risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and smoking can contribute to its development.

¹⁰ Heteroplasmy is the presence of a mixture of more than one type of an organellar genome (mitochondrial DNA (mtDNA) or plastid DNA) within a cell or individual. It is a factor for the severity of mitochondrial diseases. Since most eukaryotic cells contain many hundreds of mitochondria with hundreds of copies of mtDNA, it is possible and indeed very frequent for mutations to affect only some mitochondria while others are unaffected. Heteroplasmy can be beneficial rather than detrimental insofar as centenarians show a higher than average degree of heteroplasmy.

Mitochondrial Heteroplasmy may be intra- and inter-cellular in nature. Interestingly, such a phenomenon enlightens the location of HP zones, as well as the diverse progression of CVD either in a single patient, or in different atherosclerotic individuals.

¹¹ The acid sphingomyelinase - that is particularly active in environment with acid pH - is ubiquitous, that is to say, it is a physiological enzyme, which, however, performs its maximum activity in acidosis conditions, hence the term ASMasi

¹² At level of intima the environment has become acid in the early stages of atherogenesis, i.e., after the first year of life, and in very limited locations.

¹³ Öörni K et al. refer only to the acid sphingomyelinase, normally present in all, not mentioning the other isoforms of sphingomyelinase, which are ubiquitous enzymes,, i.e., present in all cells with a nucleus.

¹⁴ In following we summarize only some of these signs, which permit to assess, in a refined way, microvessels function and structure, including the so-called *vasa vasorum*: 1) the mean-intense (*not maximum*) digital pressure, applied upon a finger-pulp of an individual psycho-physically relaxed and in supine position, brings about upper ureteral reflex (the upper ureteral third dilates), which informs about Arterio-Venous Anastomoses (AVA) type II, group B, according to Bucciante. At this moment, if digital pressure increases *maximally*, in healthy, the reflex disappears completely, showing the structural-functional normality of these haemoderivative components, essential in regulating microcirculatory blood-flow. On the contrary, in diseased patients the reflex intensity lowers, without disappearing; 2) analogously, the mean ureteral reflex (the mean ureteral tract dilates) shows an identical behaviour under the same experimental conditions, as far as EBD are concerned; 3) the mean-intense pressure – as above described - brings about the upper ureteral reflex (See point 1), which indicates the opening of AVA type II, group B. However, if the individual, at this point, raises his arm to vertical position, reflex rapidly disappears physiologically: closure of haemoderivative formations and consequently increase of blood supply to capillaries and post-capillaries venules, aiming to preserve the physiologic histangic pH; 4) under identical conditions, in health, if the subject to examine lowers his arm vertically, the upper ureteral reflex intensity increases promptly: AVA type II, group B, dilates further and, then, their haemoderivative function increases, once more aiming to keep microcirculatory blood-flow supply in normal, physiological ranges. These physiological reactions give prominence to the normality of venous-arteriolar reflex (VAR); 5) the mean-intense digital pressure on a finger pulp, under above illustrated condition, causes gastric aspecific reflex after latency time of about 10 sec. In health, this parameter value persists unchanged in all three positions (horizontal, high vertical and low vertical), due to above-illustrated reasons. All these dynamic tests result abnormal, and of different degree, of course, in case of arteriosclerosis, starting from the very initial stage: i.e., arteriosclerotic constitution as well as its dependent Inherited real Risk.

¹⁵ During “*boxer’s test*”, which brings about vessels dilation, due to increase of the peripheral arterial resistance, increasing contemporaneously both vasomotility and vasomotion of related *vasa vasorum*, quantified by QBS, as well known to skilled reader: the intensity of artery-“in toto” ureteral reflex appears practically doubled, in healthy, and latency time of artery-caecal reflex clearly extended (= temporarily decreased tissue acidosis, due to Valsalva’s manoeuvre).

¹⁶ The subject to be examined is invited to perform Valsalva’s manoeuvre (increase of acethyl-choline) for about 10 seconds; then, doctor assesses the value of same parameter for a second time. In healthy, the intensity of both “in toto” ureteral reflex and gastric aspecific reflex doubles or augments significantly. Clinical evidence shows that the severity of arteriosclerosis and decreased intensity of “in toto” ureteral and gastric aspecific reflex during Valsalva’s manoeuvre are inversely related.

¹⁷ The following remedies and healthy lifestyles belong to the class of ‘type A’ or ‘green’ therapy: etymologically speaking diet, i.e., Mediterranean diet and physical activity, histangioprotectors i.e., conjugated-melatonin.

¹⁸ The following treatments belong to the class of ‘type B’ or ‘blue’ therapy: quantum therapy with Extremely High Frequencies (EHF) microwaves able to capture and re-transmit customized frequencies from the human body in Body Resonance Recording (BRR) mode, thermal sulfuric water.