The Glycocalyx Bedside Evaluation Plays A Central Role in Diagnosing Type 2 Diabetes Mellitus and in its Primary Prevention

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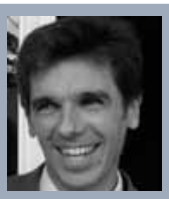
Overview

Mitochondria dysfunctions,[1-2] blood-brain barrier impairments,[3-5] endothelial[6-8] and glycocalyx evaluations[9-11] are novel insights into the pathogenesis and early diagnosis of type 2 diabetes mellitus (T2DM). T2DM is characterised by a localised endothelial cell dysfunction that underlies the development of both the micro- and macrovascular complications of the disease. Various theoretical models and experimental approaches provide data about changes to the structure and functions of the glycocalyx under various types of inflammatory conditions.[8] These alterations are suggested to promote inflammatory processes in the vessels and to contribute to the pathogenesis of a flurry of diseases, such as T2DM,[9] which is associated also with an increased vascular permeability.[10]

The normal endothelial glycocalyx is composed by glycosaminoglycans (GAGs), proteoglycans and glycoproteins fully integrated into a functional layer attached to the vascular endothelial luminal surface. The shredding of the glycocalyx appears as an essential initial step in the pathophysiology of atherosclerosis and microangiopathic complications of T2DM: the degradation of the glycocalyx enables an abnormal protein filtration at glomerular level and a progressively worsening disorder of the endothelium. These abnormalities underlie both the appearance and progression of the diabetic micro-angiopathy (DMA).[11] A number of early microvascular changes with loss of the glycocalyx, before clinically apparent vascular complications, were observed in a population of children affected by type 1 diabetes mellitus.[12] These results disclose the glycocalyx as a possible monitoring measurement for earlier detection of DMA and may provide a basis for new diagnostic and therapeutic strategies aiming at protection or restoration of the glycocalyx. This reinforces the importance, starting from the first decade of life, of a proper and timely assessment of the glycocalyx and the microvascular compartment. In the present paper we explore a pathophysiology of T2DM resumed in five-stages suggested by a clinical diagnosis, based on the Auscultatory Percussion (AP) of organs and viscera,[13-16] and termed Quantum Biophysical Semeiotics (QBS). This novel technique allows an early assessment of the clinical and preclinical stages of T2DM. Our research is based on a case series observational study run by the senior Author, between 1982 and 2012. The inclusion criteria were the patient's age (< 40 y.o.), an already established clinical T2DM diagnosis and the absence of the clinical signs of the DMA. The exclusion criteria were the patient's age (> 40 y.o.) and the presence of already well known complications related to the DMA. A total of 250 patients were recruited and evaluated using the QBS diagnostic method based on the spatial (cm) and temporal (sec) reflexes' parameters which can be observed during the AP. The following outcome measures were adopted: (1) to establish the variations over time (mean follow-up 10 years) of the parameters appreciated during



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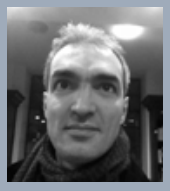

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AP and (2) to assess whether the observed numerical values over time can be correlated with the appearance and progression of the DMA from a preclinical to a clinical stage. Since the numerical values observed during the follow-up correlate with the appearance and progression of the DMA, we therefore speculate that the application of QBS clinical tests may allow an early detection of the microvascular alterations in patients with T2DM before clinically obvious vascular complications are established, thus providing useful information for an effective pre-primary and primary prevention. The alterations of QBS tests' parameters observed in our patients' population during the follow-up also prompted us to recapitulate these numerical findings into a novel classification of T2DM pathophysiological stages.

'Quantum-Biophysical-Semeiotics' Five Stages of T2DM

There is a five-stages classification underlying the pathophysiology of T2DM focused on the metabolism of glucose and the interplay between β-cell function and insulin resistance.[17] Instead, our classification[18] is focused on the biological systems' alterations, bedside evaluated. According to our experimental evidence, we speculate an association between (1) the reflexes' parameters of QBS tests, (2) the appearance and progression of the microvascular and glycocalyx abnormalities and therefore (3) the overall clinical progression of T2DM. The data obtained from QBS tests as applied to the evolution in time of the pathophysiology of T2DM can be resumed in five stages (see Table 1).

The observed parametric values[22] allow, even on the newborn, the "Diabetic Constitution" (DC) diagnosis.[23] All diabetic patients, when examined using QBS tests, are positive to "Dyslipidemic" and DC, and therefore they carry their related Inherited Real Risks (IRR), conditio sine qua non of T2DM, which, in turn, is based on a mitochondrial functional cytopathy, termed 'Congenital Acidosic Enzyme-Metabolic Histangiopathy' (CAEMH). CAEMH, under the negative influence of well-known environmental risk factors, causes tissue acidosis which, if persistent and prolonged in the islets of Langerhans,[24-26] will evolve towards the pre-metabolic and metabolic syndrome.[27] In our observational study we also noted that those individuals, whose parents were both positive for CAEMH and DC, suffered from diabetic IRR.

Stage	QBS Diagnosis / Complications	Time
1st Stage	Diabetic and Dyslipidemic Constitution, Inherited Real Risk (IRR) of T2DM — overt, latent or potential	Since birth
2nd Stage	Abnormal synthesis of perivascular GAGs by fibroblasts, pericytes, mioblasts, megacariocytes, etc; Amiline in the Interstitial Fundamental Substance, glycocalyx malfunction in both beta-cells and peripheral target-organ cells. Location: Capillaries, Small Arteries, Arterioles, AVA type II, group B, cutaneous, Endoarteriolar Blocking Devices (EBDs), malfunctioning.	About 1st decade of life
3rd Stage	IRR of T2DM, Microalbuminurie, Initial ATS plaques, etc.	About 2nd decade of life
4th Stage	Pre-diabetes, overt microvascular complications — OGTT, Iper-Insulinemic-Normo-Glicemic Clamping, Insulinemia.	About 3rd decade of life
5th Stage	Overt T2DM	About after 3rd decade of life

Table 1. The five stages of T2DM pathophysiology according to microvascular and parenchymal QBS diagnosis.

In positive DC patients, the modifications affecting all the insulin target organs are the expression of an alteration affecting the Fundamental Amorphous Substance (FAS), whose impairment starts from the second stages (it may be occasionally absent in its early part) and it is characterised by the altered composition of the glycocalyx, which is pathologically correlate with the abnormal numerical values observed in QBS tests.

The first decade of life (see Table 1) is characterised by an initial impairment and progressive abnormalities not only of the glycocalyx of pancreatic β-cells but also of the cells of the target organs of insulin. We speculate that the aforementioned abnormalities are the underlying pathophysiological substrate, which may account for the progression and worsening, over time, of QBS tests' numerical parameters. In fact, during the second stage, according with QBS diagnosis, the modified dynamics of microcirculatory microvessels are characterised by a slight microcirculatory activation[28] that show parametric values significantly different from the microvascular oscillations which can be observed in healthy subjects. For this reason, QBS tests, when applied throughout from the very beginning to the end of the second stage, offer a realistic assessment of the overall "potential pathological load" of T2DM.

At the very early phase of the second stage, only an alteration in the structure and function of the glycocalyx is present and the pathological mesenchymal expressions of the 'Tissue Microvascular Units' [29] (including FAS) are not yet established. However, these subtle pathological changes can be appreciated by QBS diagnosis.

In physiological conditions, the pericapillary network is an essential and integral part of the "periangium": the pericapillary network, adhering to the endothelium and wrapping around the capillary network, must be considered as "tissue" because it is in continuation with the reticular fibers and with the interstitial and pericellular collagen fibers of the "fixed" mesenchymal networking elements. In this pericapillary networking a special role is played by the pericytes: these cells are adherent to the endothelium but their origin is from the mesenchymal cells in the tissue surrounding the endothelium. The clinical changes observed during the pre-pathological conditions seem to confirm this origin of pericytes, precisely from the primitive pluripotent mesenchymal cell. According to Curri,[30] the endothelial

impairment would send "messages" to the primitive mesenchymal cells, which in turn differentiate generating pericytes that, with restorative purposes, will migrate so as to adhere to the capillary wall.

Outside of the pericapillary network, but close to it, there is the interstitial connective matrix constituted by the FAS and numerous and different cells (such as fibrocytes, fibroblasts, megakaryocytes, histiocytes, monocytes, etc.) which, surrounded by mesh of connective fibers, produce and synthetise FAS and GAGs.

GAGs have got an acidic nature and they can be sulfated (S-keratan, chondroitin-4 and -6 S, heparan-S, etc.), while hyaluronic acid, very hydrophilic, is non-sulfated. It has been calculated that from 200 to 500 mL/g of water can bind to GAGs.[31]

The FAS plays an important role in modulating the physiological activity, permeability and vasomotion of the pericapillary network and therefore of the capillary system too. The FAS' functions allow the solutes (i.e., water, hormones, including insulin, metabolites, parenchymal cells products, etc.) to transit from the microvessels lumen towards the parenchyma (or vice-versa). The physiological state of both the pericapillary network and the FAS can be evaluated through a systematic follow up of QBS tests' parameters, from the first stage (see Table 1). The consequences of CAEMH affect not only the parenchymal cells but also those cells functionally related to the parenchyma and/or involved in the synthesis of GAGs. An intense CAEMH is associated with an extensive microvascular remodeling caused by two mechanisms, namely (a) the orientation of the microcirculation structure by the local parenchyma[32] and (b) the modification in FAS composition. When and where the hyaluronic acid content decreases and the sulfated acid GAGs content increases, the structure of the microcirculatory walls is altered as well as the FAS' 'bound water'/'free water' ratio, the vasomotion, the interstitial transport of many metabolites, hormones, insulin, catabolites, jons, etc. In this scenario, a special emphasis should be given to the abnormal transit of insulin and lipids and the pathological structure and dynamic function of microvascular wall.[28-29]

The second stage is thus characterised by the enlargement of the interstitial space, caused by an abnormal synthesis of local GAGs, when the production of jaluronic acid appears particularly compromised. As a consequence, the ratio bound water/free water results altered, i.e., abnormally high: the clinical QBS microvascular diagnosis[28] shows that the velocity of microcirculatory wave fluctuations in Langherans's islets is particularly slow (this is also partly due to the deposit of Amilin), so that the blood-flow is significantly reduced.

The FAS plays a central role in both microcirculatory vasomotion, and permeability, as well as in the physiological transport of numerous substances from the microcirculatory bed to the parenchyma, and vice-versa.[29,33] The compromised transport of insulin, brought about by GAGs altered ratio, seems to play a central role in "insulin-resistance", typical of T2DM third stage; ATS plaques appear from the second decade of life.

The last two stages, pre-diabetes and overt T2DM, can be clinically recognised and therefore easily diagnosed with the aid of the current medical technology available.

Diagnosis: Diabetic Constitution and Inherited Real Risk of T2DM

QBS clinical tests[15-16,22-23,34] allow the clinical and pre-clinical diagnosis of the most severe diseases, i.e., solid and liquid forms of cancer, coronary heart diseases, as well as the IRR of T2DM, i.e., through the 'Osteocalcin Test'[18] and the 'Insulin Secretion Pick Test',[36] useful in assessing the impairment of the vasomotility and the vasomotion and the possible presence of the typical pathological EBDs.[28-30,33-34,37] The physician can evaluate the mitochondria functions[35] as well as the behavior of any biological system and recognise and quantify the presence of DC,[23] T2DM IRR, or overt T2DM, even if asymptomatic. The T2DM IRR is characterised by a microcirculatory remodeling, paralleling mitochondrial remodeling, due to the functional and structural abnormalities above mentioned.

Pre-primary and Primary Prevention of T2DM

QBS tests can be applied also before and during the administration of any preventive therapy for therapeutic monitoring. In our experience, focused on T2DM primary prevention, the Mediterranean Diet, as well as the administration of CoQ10, melatonin and carnitine gave satisfactory preliminary results.[26, 38, 39] The efficiency of the combination of the afore-mentioned treatments can be easily assessed comparing the variations, observed in time, of QBS reflexes' parameters, which allow the physician to make inferences regarding the status of the tissue acidosis, the tissue oxygenation[40] or the mitochondrial activity as well. Moreover, millimeter waves applied on heart[41] and pancreatic[26] trigger points, give evidence of an improvement of the mitochondrial and endothelial function, showing the existence of a genetic feedbacks which is able to heal the DC and the T2DM IRR (if the treatment is given in time i.e. before the onset of a clinically evident pathology).

Current and Future Developments

In our paper an original pathophysiological framework of T2DM according to QBS methodology is presented. T2DM is subdivided into five chronological stages. Each stage is characterised by highly precise QBS reflexes' parameters values, which can be correlated with the progression of T2DM from a preclinical (asymptomatic) to a clinically evident stage. A certain bedside diagnosis of the QBS pre-clinical T2DM, can be easily recognised even from birth: if this were done during the first decade of life on large populations of patients, an intelligent prevention strategy could be implemented only on those individuals at IRR of T2DM.

References

1. Supale S, Thorel F, Merkwirth C, Gjinovci A, Herrera PL, Scorrano L, Meda P, Langer T, Maechler P. Loss of prohibitin induces mitochondrial damages altering β-cell function and survival and is responsible for gradual diabetes development. Diabetes. 2013 Oct;62(10):3488-99.
2. Khan S, Raghuram GV, Pathak N, Jain SK, Chandra DH, Mishra PK. Impairment of mitochondrial-nuclear cross talk in neutrophils of patients with type 2 diabetes mellitus. Indian J Clin Biochem. 2014 Jan;29(1):38-44.
3. Yuan SY, Ustinova EE, Wu MH, Tinsley JH, Xu W, Korompai FL. Protein kinase C activation contributes to microvascular barrier dysfunction in the heart at early stages of diabetes. Circ Res 2000 Sep 1;87(5):412-7.
4. Sajja RK, Prasad S, Cucullo L. Impact of altered glycaemia on blood-brain barrier endothelium: an in vitro study using the hCMEC/D3 cell line. coronary exchange vessels in early diabetes.Fluids Barriers CNS. 2014 Apr 5;11(1):8.
5. Serlin Y, Levy J, Shalev H. Vascular pathology and blood-brain barrier disruption in cognitive and psychiatric complications of type 2 diabetes mellitus. Cardiovasc Psychiatry Neurol. 2011;2011:609202.
6. Fandiño-Vaquero R, Fernández-Trasancos A, Alvarez E, Ahmad S, Batista-Oliveira AL, Adrio B, Fernández AL, González-Juanatey JR, Eiras S. Orosomucoid secretion levels by epicardial adipose tissue as possible indicator of endothelial dysfunction in diabetes mellitus or inflammation in coronary artery disease.Atherosclerosis. 2014 May 21;235(2):281-288.
7. Polovina MM, Potpara TS. Endothelial dysfunction in metabolic and vascular disorders. Postgrad Med. 2014 Mar;126(2):38-53. doi: 10.3810/pgm.2014.03.2739. Review.
8. Gilbert RE. The endothelium in diabetic nephropathy. Curr Atheroscler Rep. 2014 May;16(5):410.
9. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators Inflamm. 2014; 2014:694312.
10. Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, Schlingemann RO, Nieuwdorp M, Stroes ES, Vink H. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia. 2010 Dec;53(12):2646-55.
11. Frati Munari AC. Medical significance of endothelial glycocalyx. Part 2: Its role in vascular diseases and in diabetic complications. 2014, Arch Cardiol Mex. 2014.
12. Nussbaum C, Cavalcanti Fernandes Heringa A, Mormanova Z, Puchwein-Schwepcke AF, Bechtold-Dalla Pozza S, Genzel-Boroviczény O. Early microvascular changes with loss of the glycocalyx in children with type 1 diabetes. J Pediatr. 2014 Mar;164(3):584-9.e1.
13. Guarino JR. Auscultatory percussion of the head. Br Med J. 1982 Apr 10;284(6322):1075-7.
14. Guarino JR. Auscultatory percussion of the bladder to detect urinary retention. N Engl J Med. 1981, 305(12) 701.
15. Stagnaro MN, Stagnaro S. Early clinical diagnosis of osteoporosis using auscultatory percussion. Therapeutic monitoring Clin Ter. 1991 Apr 15;137(1):21-7.
16. Marchionni M. The Auscultatory Percussion of the Stomach Plays a Central Role in Bedside Diagnosis and Primary Prevention of Neurodegenerative Diseases and their Inherited Real Risks. BIT's 5th Annual World Congress of Neurotalk 2014. May 16-18, 2014. Nanjing, China. Available at: http://www.sisbq.org/uploads/5/6/8/7/5687930/nanjing_marchionni2014.pdf
17. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. 2004, Diabetes 53(Suppl. 3), S16–S21.
18. Stagnaro S, Caramel S. Inherited Real Risk of Type 2 Diabetes Mellitus: bedside diagnosis, pathophysiology and primary prevention. 2013, Front. Endocrinol. 4:17.
19. Caramel S, Stagnaro S. The role of glycocalyx in QBS diagnosis of Di Bella's Oncological Terrain. 2011, JOQBS. http://www.sisbq.org/uploads/5/6/8/7/5687930/oncological_glycocalyx2011.pdf
20. Caramel S, Stagnaro S. QBS of Oncological IRR of Myelopathy: the diagnostic role of glycocalyx. 2011, JOQBS. http://www.sisbq.org/uploads/5/6/8/7/5687930/qbs_myelopathy_glycocalyx_english.pdf
21. Caramel S, Stagnaro S. Skeletal Muscle Cell Glycocalix Evaluation during CFS Treatment corroborates Andras Pellionisz's Recursive Fractal Genome Function Principle . 2011, JOQBS. http://www.sisbq.org/uploads/5/6/8/7/5687930/cfsglycocalyx.pdf
22. Caramel S. Auscultatory Percussion of the Stomach, according to Sergio Stagnaro. Handbook. 2014, QBS press. Available at: http://www.sisbq.org/uploads/5/6/8/7/5687930/cardiogarap_handbook.pdf
23. Stagnaro S. Siniscalchi's sign. Bedside recognizing, in one second, 'Diabetic Constitution', its 'Inherited Real Risk' and Type 2 Diabetes Mellitus. 2010, JOQBS. Available at: http://www.sisbq.org/uploads/5/6/8/7/5687930/siniscalchi_sign.pdf
24. Stagnaro S., Stagnaro-Neri M. Le Costituzioni Semeiotico- Biofisiche. Strumento clinico fondamentale per la prevenzione primaria e la definizione della Single Patient Based Medicine. (2004). Roma: Travel Factory.
25. Caramel S, Stagnaro S. Vascular calcification and Inherited Real Risk of lithiasis. 2012, Front. Encocrin. 3:119.
26. Stagnaro S, Caramel S. The role of modified mediterranean diet and quantum therapy in type 2 diabetes mellitus primary prevention. 2013, JPNAS 3, 59–70.
27. Stagnaro S, Singh RB. Influence of nutrition on pre-metabolic syndrome and vascular variability syndrome. 2009, Open Nutr. J. 2.
28. Stagnaro S, Caramel S. The Key Role of Vasa Vasorum Inherited Remodeling in QBS Microcirculatory Theory of Atherosclerosis. 2013, Front. Genet. 4:55.
29. Stagnaro-Neri, M., and Stagnaro, S. Introduzione alla Semeiotica Biofisica. Il Terreno Oncologico. (2004) Travel Factory: Roma.
30. Curri, S. B. Le Microangiopatie. (1968) Milano: Inverni della Beffa.
31. Zenda T, Murase Y, Yoshida I, Muramoto H, Okada T, Yagi K. Does the use of insulin in a patient with liver dysfunction increase water retention in the body, i.e. cause insulin oedema? 2003, Eur. J. Gastroenterol. Hepatol. 15, 545–549.
32. Stagnaro S. Quantum biophysical semeiotics: the theory of angiobiopathy. 2009. Available online at:http://wwwshiphusemeioticscom-stagnaro.blogspot.it/2009/05/quantum-biophysical-semeiotics-theory.html
33. Ditzel J. Functional microangiopathy in diabetes mellitus. 1968, Diabetes 17, 388–397.
34. Stagnaro S, Stagnaro-Neri M. Valutazione percusso-ascoltatoria del Diabete Mellito. 1986, Aspetti teorici e pratici. Epat. 32, 131.
35. Saotome M, Hajnóczky G, Katoh H, Satoh H, Hayashi H. "Mitochondrial remodeling" in coronary heart disease. Research Reports in Clinical Cardiology, 12 June, 2014, 111, 122.
36. Stagnaro-Neri M, Stagnaro S. Semeiotica Biofisica: valutazione clinica del picco precoce della secrezione insulinica di base e dopo stimolazione tiroidea, surrenalica, con glucagone endogeno e dopo attivazione del sistema renina-angiotesina circolante e tessutale.1997, Acta Med. Medit. 13, 99.
37. Stagnaro, S. Diet and risk of type 2 diabetes. 2002, N. Engl. J. Med. 346, 297–298.
38. Stagnaro-Neri M, Stagnaro S. Sindrome di Reaven, classica e variante, in evoluzione diabetica. Il ruolo della Carnitina nella prevenzione del diabete mellito. 1996, Il Cuore 6, 617.
39. Stagnaro S. A Clinical Way in Fighting the Five Stages of Type 2 Diabetes Mellitus. 2011.Available online at:http://www.biomedcentral.com/1741-7015/9/76/comments
40. Ditzel J, Standl E. The problem of tissue oxygenation in diabetes mellitus. I. Its relation to the early functional changes in the microcirculation of diabetic subjects. 1975, Acta Med. Scand. Suppl. 578, 49–58.
41. Caramel S, Stagnaro S, Pyatakovich FA, Yakunchenko TI, Makkonen KF, Moryleva ON. Background Millimeter Radiation Influence in Cardiology on patients with metabolic and pre-metabolic syndrome. Journal of Infrared and Millimeter Waves, Shangai, China, Vol.1, February 2014.