

Scientific Report

Nutritional considerations on platelet fatty acids in Major Depression and Ischemic Cardiovascular Disease

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Abstract

In our study we have evaluated the theme of the platelet fatty acid composition in subjects with a clinical diagnosis of Major Depression (MD), in subjects with a clinical diagnosis of Ischemic Heart Disease (IHD) and in control subjects.

As far as we know, the platelet fatty acid composition has never been analyzed before, in MD.

The results obtained with a Self Organizing Map (SOM) show the evidence of three fatty acids, Arachidonic Acid (AA), Linoleic Acid (LA), and Palmitic Acid (PA) in a peculiar position with respect to the biochemical characterization of MD and three fatty acids, Arachidonic Acid (AA), Linoleic Acid (LA) and Oleic Acid (OA) in a peculiar position with respect to the biochemical characterization of the IHD.

Nutritional considerations are done about the possibility of a positive modification of the platelet fatty acids in both pathologies.

Key Words: Platelet, Depression, Ischemic Heart Disease, Fatty Acids, Self Organizing Map, Nutrition.

Introduction

It is increasing the awareness that psychiatric diagnosis is performed without using any objective diag-

nostic biological tool, and, very often, the diagnosis of Major Depression is misleading.

Psychiatry seems to be one of the few medical disciplines, maybe the only one, which doesn't exercise any instrument except scales of evaluation and, often, diagnosis and therapy are entrusted to the clinical experience of the psychiatrist.

There have been countless attempts and proposed solutions to this problem but there are not known methods, easy and economic, that can be used in vivo to help the psychiatric diagnosis.

Nevertheless during the past two or three decades many important researches have been performed to find biological approaches to the psychiatric diagnosis.

Many assumptions have been made about the existence of different neuro chemical aspects both, between the late and early onset of depression and within the same pathology. [1]

Further, the literature has suggested considering platelets as a key district [2, 3, 4, 5, 6, 7, 8].

From these knowledges and considerations, aroused the idea to investigate the fatty acid composition of platelets, in their integrity, researching for a new approach which could give a picture of the platelets lipid assessment in psychiatric disorders and Ischemic Heart Disease. Having realized that the traditional and advanced statistical approach was involving many fatty acids between platelets of normal, depressed and ischemic people, and seeming a complex problem, advanced mathematical tools were used. [9, 10, 11, 12]

In particular it seemed more appropriate the use of an Artificial Neural Network (ANN): the Self Organizing

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Map (SOM) described by Kohonen (Kohonen Network). [13], we called the map of depression ADAM and the map of ischemic heart disease CAIN.

As a result the SOM isolated only three fatty acid (Arachidonic Acid-AA-, Linoleic Acid-LA- and Palmitic Acid-PA-) and (Oleic Acid-OA-, Linoleic Acid-LA- and Arachidonic Acid-AA-) among all, and was able to map

the different populations respectively, normal and depressive and normal and ischemic, recognizing as similar those belonging to the same population and, in the meanwhile, different, those belonging to one population, from the other ones.

Results of the study

The results gave the following tables and figures for Major Depression and for Ischemic Heart Disease. Table 1, 2; Figure 1, 2.

Table 1: Platelet fatty acids in normal and depressive

Fatty Acids	Normal (average±SD)	Depressive (average±SD)	p
C 14:0	0.87±0.59	1.030.706	N.S.
C 16:0	20.68±2.15	17.92±4.462	< .01
C 16:1	1.48±0.71	2.02±1.571	< .05
C 17:1	0.80±0.540	0.45±0.267	< .01
C 18:0	11.22±3.00	12.7±3.016	< .01
C 18:1 n9	22.19±2.08	21.14±4.134	N.S.
C 18:1 n7	1.82±0.64	1.89±0.870	N.S.
C 18:2 n6	19.40±2.69	16.71±3.359	< .01
C18:3 n3	0.48±0.17	0.73±1.554	N.S.
C 20:3 n3	2.11±0.76	2.29±0.773	N.S.
C 20:4 n6	14.06±2.41	19.03±3.839	< .01
C 22:4	1.62±0.704	1.60±0.820	N.S.
C 22:5	1.16±0.615	0.98±0.564	N.S.
C 22:6 n3	2.09±0.80	1.49±0.802	< .01

Table 2: Platelet fatty acids in normal and ischemic subjects

Fatty Acids	Normal (average±SD)	Ischemic (average±SD)	p
C14:0	0.87±0.59	0.34±0.26	< 0.01
C16:0	20.68±2.15	23.32±3.17	< 0.01
C16:1	1.48±0.71	0.74±0.54	< 0.01
C18:0	11.23±3.00	17.65±2.50	< 0.01
C18:1 n9	22.19±2.08	17.48±2.14	< 0.01
C18:1 n7	1.82±0.64	1.04±0.46	< 0.01
C18:2 n6	19.41±2.69	10.51±3.44	< 0.01
C18:3 n3	0.48±0.17	0.59±0.30	< 0.05
C20:3 n3	2.11±0.76	0.73±0.42	< 0.01
C20:4 n6	14.06±2.41	15.17±3.01	< 0.05
C22:6 n3	2.09±0.80	1.87±0.70	N.S.

Figure 1: Distribution of the subjects (green=normal, red=depressive) over the SOM.

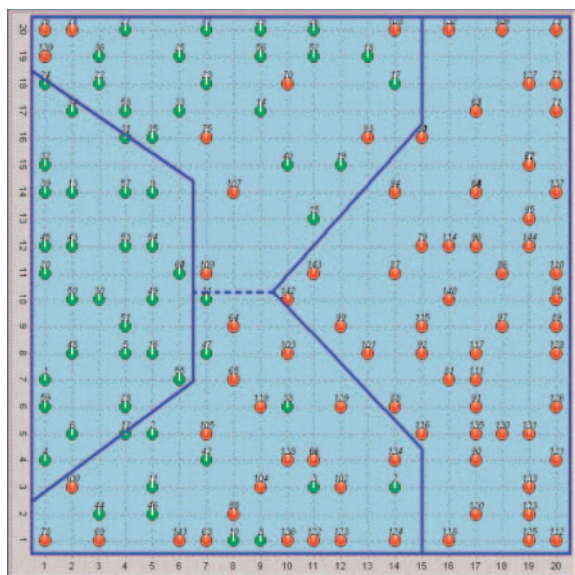
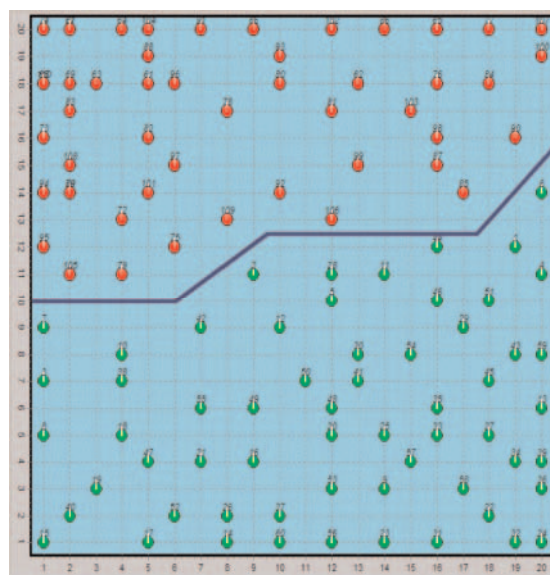


Figure 2: Distribution of the subjects (green=normal, red=Ischemic) over the SOM.



The experimental approach was very far from the concepts of the Evidence Based Medicine (EBM). In fact we want to photograph the two pathologic populations as such, and try to find commonalities and differences.

Then it has been considered appropriate the choice of samples beyond age, sex and therapy as already reported by Tiemeier [14].

The concept was: or it is possible to find markers of general value or the method would have been limited in scope results and, perhaps, misleading.

In any case the main goal of the method was not to merely find bio markers of the disease, but which were the biomarkers that could find, overall, the differences between the populations investigated.

Further if the platelet target was really a good tissue to study the depressive and ischemic bio markers.

The direct task to find bio markers according to the rules given by the EBM, requires the elimination of selection bias and leads to a selectivity often not likely, usually unrealistic.

The SOM solution, at least in our case, allowed performing the analysis regardless.

This result is receiving a considerable consensus from the international scientific world as well as giving an important contribution to open the door to the understanding of possible biochemical dynamics underlying the diseases.

This could represent a new approach to the problem, indeed, having joined:

- 1) the platelets fatty acid content, as synthetic but complete source of information
- 2) a mathematical analysis using a non-linear self-organizing system

This allows better exploring the informations contained in platelets which, in their tests, have confirmed to be the "ambassador" of the neuron.

There is a third, crucial key within the searches conducted so far, coming from the intrinsic characteristics of the platelet and the mathematic algorithm used.

The method proposed "photographs" the populations, as they are, and tries to find commonalities and differences.

In other words, it is as if the subject is considered in its entirety and complexity, beyond variables that, naturally, differ from individual to individual.

The first fundamental consequence, is that it derives a diagnostic tool extremely effective, practical and with a strong tolerance to "noise". Secondly, the choice of specific fatty acids and their relative strength in the classification made by the SOM allows investigating the problem and helps in understanding the disease, from the biochemical, and also the nutritional point of view.

At present, the method has proved its effectiveness particularly in the study of brain-related diseases, allowing, in fact, reaching remarkable results, and obtaining confirmations of different nature in the checks carried out. Some of the depressive subjects are mixed, in the SOM, and this can correspond to misleading diagnosis of Major Depression [15]

The results obtained so far, indicate the possibility that the method can be extended to other psychiatric conditions.

The diagnostic tool for Ischemic Heart Disease has its topic in a practical and immediate possibility to early diagnose the plaque formation. In this area the researchers had numerous confirmations until to get a compatibility of the result with the well known Framingham Score [16]. Further investigation has even allowed assessing commonalities between the depressive disorder and heart disease which, in accordance with the current scientific literature, appear to be related. [17]

Ischemic disease, together with Depression, is recognized to be among the main causes of pathology and death in the next future, according to the indications of the World Health Report 2002.

Nutritional Considerations

The scientific literature has often expressed itself on the relationship between nutrition and health. Numerous scientific works describe, for example, the modifications in the lipid moiety of the cellular membranes under the influence of the different nutritional lipids, many works concern research into the intervention of certain nutrients, on the multiple cellular functions, on some biochemical paths of metabolic transformation, on some crucial points of the production of cytokines and eicosanoids.

The recent experiences performed on the evaluation of the platelet fatty acids have led to think that the possible nutritional modifications induced from the outside are not really so significant. Perhaps the moment has come to critically reconsider the relationship between

nutrition and health beyond the *deja vu* of the literature that, often, is handed down from decade to decade, beliefs amplified by experimental impressions.

Certainly the chapter on the relationship between nutrition and health is a complex one in the life of man, but at the same time a very simple one too. If we consider the mathematical rules that govern the animal biological system, in particular that of man, we realise that the fundamental food-man relationship occurs on the basis of an energy regulation whose fundamental purpose is the maintenance of the biochemical-metabolic-function homeostasis.

On this principle all of the reactions of the organism are developed with stochiometric precision guaranteeing the proper functioning of the most complex and perfect machine ever built.

The questions we must ask ourselves then are:

1. If man were to respect with painstaking determination the equation nutrition- homeostasis would this produce a consequently perfect and healthy organism?
2. In reality could it avoid the so-called social pathologies that go from the cardiac ischemias to those of behaviour and the metabolic ones?

It is hard to answer these questions, for several reasons.

Certainly if everything is done properly, even the gene expression, one of the main mechanisms of interactions with the nutrients for health should function perfectly as well as all the interactions and the biochemical and metabolic functions.

What then makes it difficult to create these conditions? What happens in this food-man relationship when it goes beyond the margins that should be guaranteed by a perfect state of health? In this case, too, the answers are difficult to get.

The imperfection of this relationship, if we look at the extremes, is manifested mainly with the pathological fattiness and the pathological thinness, in the interval a whole series of possible conditions that can be affected by incorrect nutritional repercussions persistent in determining alterations in the cell, organ and apparatus functions.

It is in this interval that the right nutritional dimension should be matter of evaluation, finding that point of encounter that optimises the two extremes and this does not seem to be difficult.

Instead, more complex seems to be those aspects that are certainly subtle and that often the popular imagination, and not just that, link to nutrition.

Let us take, for example, atherosclerosis, a pathological condition that harbors many problems; could it be perhaps be true that it is linked to the nutrition or might it be true that the nutrition finds a favorable substrate on which to make a possible nutritional error prevail?

If we consider the recent work on the platelet fatty acids and observe what happens in children [18], it is hard to explain what can be referred to the nutrition and that the very high stearic acid level found in platelets is the result of the nutrition.

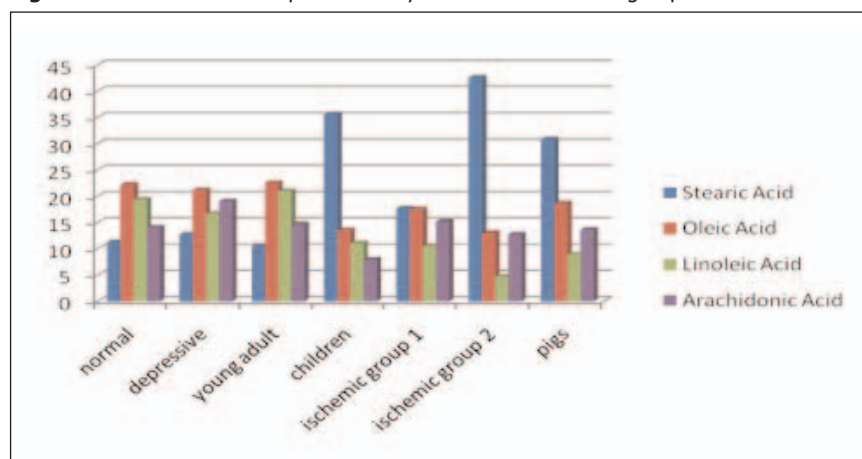
In the following figure 3 are presented the fatty acids characteristic of the SOM classification (Oleic, Linoleic and Arachidonic) and the stearic acid concentration of lipid platelets [12].

It was clear that there was a huge difference among the groups for the stearic acid concentration (Normal Subjects, Depressive Subjects and Young Adult) and the (Children, Ischemic Subjects Group 1, Ischemic Subjects Group2, and Pigs). The differences are marked in the graphs. Fig. 3.

Facing such results, observations and questions arose.

- 1) Why the first group of ischemic people investigated had a lower stearic acid with respect to the second ischemic group?

Figure 3: distribution of the platelets fatty acids in the different groups



2) Why children and pigs had a higher stearic acid with respect to the normal, depressive and young adult people?

We tried to find answers to difficult questions:

1) The stearic acid of the ischemic group 1 is lower than that one of the ischemic group 2 but is, in any case, higher than in normal, depressive and young adult. This brings to the first consideration, i.e. stearic acid platelet concentration is a characteristic of the ischemia. In the same way the ratio Stearic/Oleic acid (SI-Saturation Index) is able to classify the normal and ischemic subjects according to the SOM (CAIN). What is more difficult to explain is the difference between the two ischemic groups. The more evident difference between the two groups was linked to the therapy. The second group was under statins, hypotensive an antiaggregating therapy and the general clinical conditions were worst than those of the first group. The first group was not under massive therapy and not under statins.

Is it possible that exists two different ways to be ischemic or certain drugs, such as statins are influencing the stearic acid level? [19]

In any case the stearic acid level seems to increase as a mechanism of compensation for the stabilization of the platelet because of its activity on the same platelets. [20, 21, 22, 23, 24, 25, 26, 27]

Despite the platelet stearic acid level differences, both ischemic groups find the same position in CAIN confirming the same peculiar relationship among Oleic, Linoleic and Arachidonic Acid.

2) The explanation that we have assumed to interpret the children and pig stearic acid results is that children and pigs are young organisms and, as such, they must have a smaller amount of cholesterol in membranes [28]. The Stearic Acid, because of its positive effects on the platelets [23, 24], could represent a mechanism of compensation to control the phenomena of activation of the platelets and of a higher degree of unsaturation, protecting an unstable system, through the increase of the degree of saturation.

Why does the evolving of age towards the complete biochemical maturation remove from the platelet that substantial quantity of stearic acid that makes the difference, and why is it no longer definitely possible to

reinstate those protective levels, which actually, probably, shield the child from the ischemic cardiovascular event? Is it possible to achieve such levels of stearic acid in platelets by nutrition, in order to verify the protective effect that children benefit from?

So, is it possible to use this information to reduce the cardiovascular risk in the adult, is it possible to think of being able to influence, through nutrition, the composition of the platelet fatty acids, when we have shown that the stearic/oleic acid ratio (Saturation Index- SI) cadences the progression of age? [12]

If one, once again, considers as reference the platelet fatty acids and the evaluations of the Framingham Score in comparison with the neural network then a further consideration is possible.

As is well-known, the Framingham score expresses the 10-year risk of the ischemic cardiovascular disease. If we exclude the classical risk factors from the Framingham, a curve of cardiovascular risk remains for age (from 20 to 80 years) < 15%, that is, practically the normality curve relating to ageing. [29] Figure 4, 5, 6.

As the platelets fatty acids have highlighted the same cadence, at different ages, using the stearic/oleic ratio (SI), how can we think that the subjects are affected by nutrition beyond a certain measure? Nutrition is necessary to provide them, but it is not crucial to modify them significantly.

What are the intervals within which it is possible to influence a cellular lipid composition?

There is evidence that it is wrong to think of obtaining the modification of only one fatty acid and that this is the best maneuver, as there is the reasonable cer-

Figure 4: Framingham risk areas related to age.

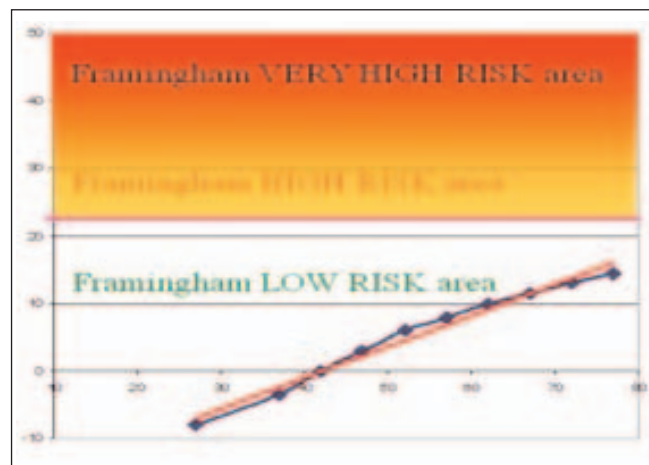


Figure 5: CAIN risk area for age according to SI (Stearic/Oleic ratio).

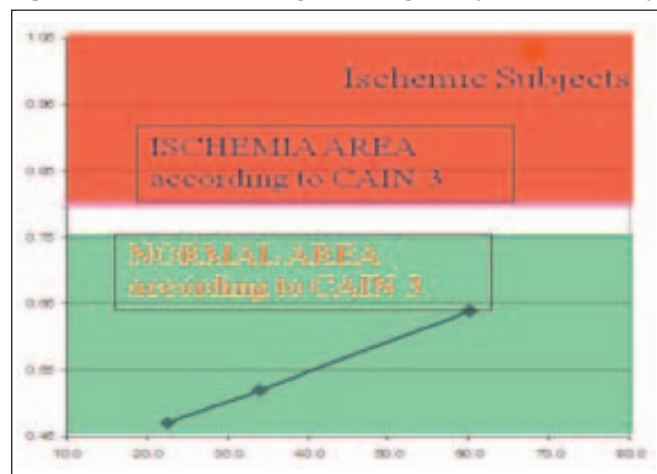
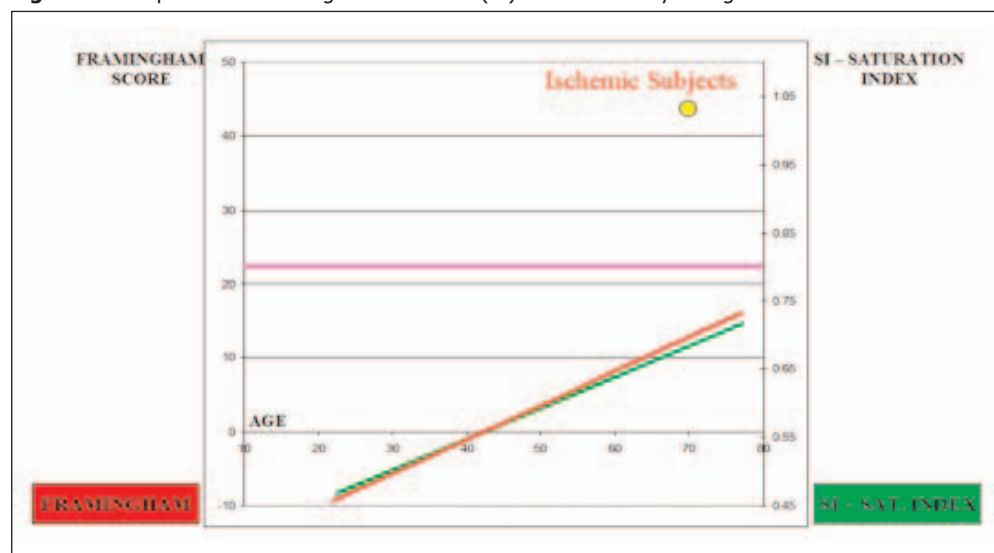


Figure 6: Comparison of Framingham and CAIN (SI) risk curves only for age.



tainty that it is in the specific combination between some platelet fatty acids that, for example, the condition of normality is played in respect to the pathological one, definitely influencing the determining of positive or negative events on the platelet cellular function.

How can we think, therefore, that certain nutritional interventions are possible?

Here then nutrition is going through a moment of difficulty, it can only be delegated the role of opting, broadly speaking, for what is healthy and using it with moderation.

For example, in the case of ischemic cardiopathy, it could be the role of olive oil owing to its substantial oleic acid content.

If, on the one hand, children defend themselves with the physiological rise in stearic acid that then collapses in adult age, perhaps, owing to the characteristics that connote the biochemical aspect of the ischemic disease (low levels of oleic acid) it is opportune to raise that level.

It is evident that such a condition will have to be carefully monitored in order to understand what the quantity of oleic acid is that we can transfer to the platelets and if it is, ultimately, possible to restore conditions of normality in the platelet function such as to reduce the size of the risk of their activation with all the consequent phenomena. The hypothesis, according to the Food and Drug Administration, whose advice is to consume at least 23 g. of olive oil by day (corresponding to about 16 g. of oleic acid), is to suggest a consumption of, at least, 30 g. of olive oil (corresponding to the average of 20 g. of oleic acid). It is with this approach that it has been demonstrated the possibility to substantially modify the platelet fatty acid composition in pigs [30].

In the studies performed, the combination between mathematics, nutrition and disease have perhaps rather than weakened a pre-existing model of thinking, determined strong convictions of unavoidable pre-

destinations in some points of the biological system (or perhaps all the points of the biological system?).

Conclusion

What conclusive argument can we hope for, in all the conditions that correspond to the positioning in the neural network of the pathological cases, and with the chance of precocious evidence, it would be worthwhile intervening pharmacologically or with nutritional solutions that protect e. g. the platelet from phenomena of hyperactivation, such as coagulation and others events?

As we cannot deny the inevitable and unequivocal relationship between food and man, the obligation results to use all the nutritional strategies useful and critical so that the organism may have correlated benefits.

In any case it is still hard to understand which are the filters through which such a provision of nutrients should pass, which are the intervals really compatible with homeostatic guarantees and what is the efficacy or the limitation of the nutritional intervention itself.

This highly innovative concept in actual fact is the consequence of a mathematical and biological evaluation of human life, as well as representing the starting point for a new thinking on the relationship between food and man to understand if it contains, per se, the prerequisites of inevitable mechanisms of adjustment and that sees gene expression and metabolic error play a dominant role in the regulation of the biological system in respect to the possible modifications induced by nutrition.

If we observe the distribution of the platelet fatty acids we immediately become aware of more evidences:

1. The sum of the major fatty acids, in membranes, represents around 90% of the whole set;
2. Their weights are well calibrated between them;
3. The major fatty acids are the same ones that the most sophisticated statistical investigations and the neural networks have selected in the characterization of the diseases undergoing investigation.

Hence, the game is fundamentally played in the shift of their equilibrium as has been substantially demonstrated by the specifically constructed SOMs.

If we take the two moments studied (i.e. Major Depression and Ischemic Cardiovascular Disease) as reference, we immediately realize that two characterizing elements emerge: arachidonic acid for depression and oleic acid for ischemia.

The other major fatty acids, that is the 16:0, the 18:2, and the 18:0 practically adjust themselves solely as a function of the two dominants, actually determine a sort of equilibrium within which the pathological event can be recognized.

However, the SOM has shown that in each pathological condition it is the relationship that the single fatty acids establish between the respective triplets that

govern the biochemical condition which, in turn, determines the platelet response.

In fact, if we simulate in the network (SOM) the shifts of the single fatty acids to modify the position of the subject with respect to the normality or the pathology, we must take into account the fact that inevitably the shift of one of the fatty acids must be matched by the shift in one or more of the others for evident reasons of occupation of the membrane space.

It is more logical to think that it may be the shift of one or more of the major ones that determines the response rather than a shift in the minor ones, seeing the total nomination of the former over the latter.

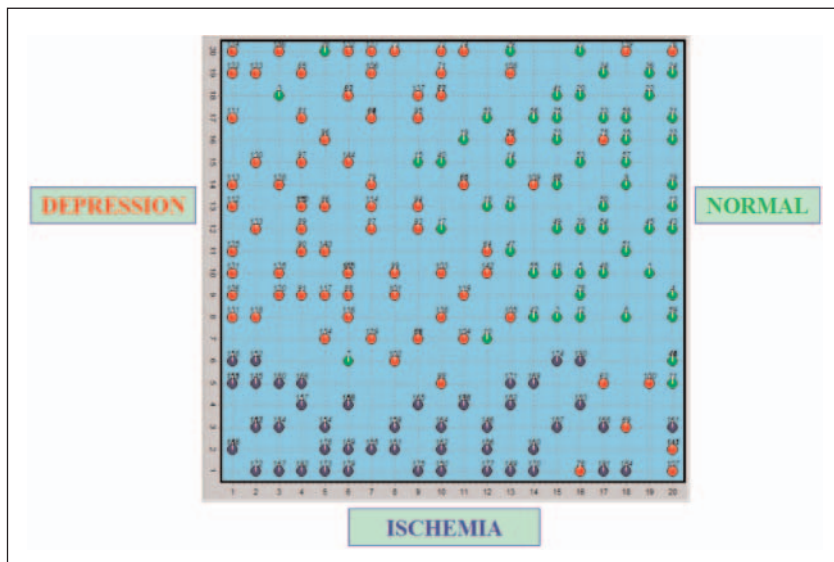
To the minor fatty acids we must think more to micro-effects than to macro-responses, that is, if there are any, to micro-regulation effects.

The shifts will have to induce new balances that the network is able to verify.

Well, the shifts required are multiple and calls for contemporaneous adaptation within each triplet to guarantee the recognition of the patients within a definite set.

To prove the great grouping capacity of the constructed SOM, the creation of the network where all three groups were inserted, grouped them again simultaneously on the basis of the characteristics of the triplets

Figure 7: In figure is evidenced the simultaneous classification, through the SOM, of the three groups of subjects (Normal, Depressive and Ischemic). In the right corner of the map ischemic and depressive subjects are mixed and have, in common, a low oleic acid.



previously highlighted and all different from one another [31]. Figure 7.

Does this complex and unequivocal mechanism of recognition of the pathological and the normal triplets guarantee the possibility to make a nutritional correction?

As it appears, we must necessarily argue that the relations within each triplet that regulate the functional responses of the platelets are in support of a condition of normality or pathology. How can it be thought that the nutritional correction of the lipid fraction is an easy task, such as to bring it back to the condition of normality?

In theory the result could be obtained by correcting, virtually, each one of the fatty acids specifically involved in order to go back to that set that guarantees normality.

What, then, remains to be done? Apart from holding more and more plausible the existence of a metabolic error consequent to or concomitant with some alteration in the gene expression, there perhaps concretely remains a possibility for intervention. As we know that a shift in the oleic acid is matched by a shift in the arachidonic acid and as they are, probably, the two main indicators of the pathologies studied, that is oleic acid for ischemia and arachidonic acid for depression; it would seem justified to try in any case for a rise in the oleic acid. It follows that the defense mechanisms that bind the oleic to the activity of the platelet could be improved in respect to the activation mechanisms of the same and on the other hand a reduction could be effected of the arachidonic acid that for the properties it has demonstrated in affecting the platelet in a depressive sense (increased membrane fluidity, increased transport of arachidonic acid to the brain, negative interaction with the secretion of serotonin, etc...) could improve the situation.

Thus, the oleic acid represents a plausible intervention for the correction of the two pathologies, keeping an eye on the movement of the other fatty acids, so that by a surplus or deficit in some of them, a new condition of disequilibrium is created that again determines the pathology.

The instrument of the neural networks in respect to what has already been identified is, perhaps, the only instrument usable today to assess in mathematical terms whether the biological nutritional event of modification of the platelet fatty acids is possible or not.

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