THE ACCURACY OF NAFLD DIAGNOSIS USING MATHEMATICAL SCORES IN ACTIVE YOUNG OBESE PATIENTS

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Abstract: The impact of the non-alcoholic fatty liver disease, under epidemiological, biological, pathogenic and social-economic aspect is tightly connected to obesity, type 2 diabetes mellitus and dyslipidemia. The prevalence of the disease has rapidly increased, in parallel to the dramatic increase of the population suffering from obesity and diabetes mellitus and to the decrease of physical activity in the daily living. One such consequence of our sedentary and excessive lifestyle is nonalcoholic fatty liver disease (NAFLD), which is now considered the most common cause of chronic liver disease. The natural history of the non-alcoholic fatty liver depends on the histological type at the time of diagnosis. "The golden standard" in the diagnosis of the lesions from NAFLD is represented by the hepatic biopsy. There are a series of disadvantages and problems in the clinical practice, related to the hepatic biopsy namely: intra and interobservational variability, considerable error rate in the absence of some patognomonical histological modifications, high cost, invasive method, low adherence of the patient, risk of complications. Some non invasive diagnosis methods can be used in the management of NAFLD patients. Up to now no non-invasive method considered separately, doesn't have enough accuracy to allow the replacement of hepatic biopsy in the certitude of NAFLD, but if we combine them, we can reduce the number of those patients who perform the biopsy to set the progression of the disease. Furthermore, interdisciplinary studies are needed to examine the individual contributions of physical inactivity and overnutrition to the escalating prevalence of NAFLD.
Keywords: *NAFLD, liver biopsy, non-invasive scores, disease progression.*

Introduction

Obesity and non-alcoholic fatty liver disease (NAFLD), two widespread diseases among young people and very closely related to each other, have a great negative impact on life, under epidemiological, biological, and social-economic aspects.

The prevalence of NAFLD has a significant increase, in parallel with the increase of obesity and diabetes mellitus. However the incidence of the disease remains unknown due to the lack of prospective studies. In 2011, Vernon and his collaborators published a review, where he reported values of the NAFLD incidence varying very much, between 31 cases in 1000 inhabitants/year in Japan, up to 29 cases in 100,000 inhabitants/year in England, so that, further studies are needed in order to establish a global incidence (1,2).

While physical activity represents a key element in the prevention and management of many chronic diseases, we and others believe that physical inactivity is a primary cause of obesity and associated metabolic disorders (3). Physical inactivity and NAFLD are intimately linked, but, as Rector et al reported in several

studies, the majority of this evidence is only associative, gathered from cross-sectional observations (4,5). Other studies also reported that decreased levels of daily physical activity were highly associated with increased incidence of NAFLD, higher intrahepatic fat content and significantly elevated liver enzymes in subjects who reported reduced habitual physical activity (6, 7,8).

The natural evolution of NAFLD, from simple steatosis to inflammation and fibrosis, depends on the histological type at the time of diagnosis, and on the risk factors. The most patients are diagnosed with simple steatosis and the disease never progress, but for some of them, the disease will progress to fibrosis and hepatic cirrhosis, under the action of known or unknown factors.(9,10,11,12).

On the other hand, obesity represents an important health problem because it increases the risk for many others diseases such as : NAFLD, high blood pressure, type 2 diabetes mellitus, stroke, obstructive sleep apnoea. In 2016, 39% of adults over 18 years were overweight and 15% were obese (650 million adults).

To establish the histopathological stage of NAFLD is necessary liver biopsy, which is the"golden standard" in diagnosis of the disease. Although it is unanimously recognised the importance of liver biopsy, it is also known that there are a lot of disadvantages and problems in the clinical practice such as: intra and interobservational variability, especially by the lack of a consensus regarding the indications of the biopsy in NAFLD [13,14,15], a great rate of error in the absence of some patognomonical histological features, high cost, invasive method, low adherence of the patient, risk of complications, including death. Due to all these reasons many reaserchers developed mathematical scores to identify the stage of the disease, using biochemical parameters. Up to now any non-invasive score considered separately, doesn't have enough accuracy to replace liver biopsy in diagnose of NAFLD, but if we combine some of them we can take a correct decision in choosing the patient who need liver biopsy. [16,17,18].

The aim of our study was to correlate the mathematical scores with liver biopsy in active, young patients diagnosed with NAFLD. Also we tried to establish a relationship between this disease and the level of physical activity. In this way by both approaches we want to identify the patients with high risk of progression of the disease, because it is necessary to focus on these patients. It is well known that the best therapeutic approach is weight loss and physical activity. Pharmacological treatment should be used only for patients with progressive disease (inflammation, fibrosis, cirrhosis).

Material and Method

In this study we included 50 young patients, with various degree of obesity, with evidence of hepatic steatosis on ultrasound or incidental finding of high level of liver enzymes. At the time of inclusion some patients had, also, a diagnostic of diabetes mellitus and some of them of metabolic syndrome. Before the recruitment in our study, all patients had been active people in previous year, according to EASL recomandations: aerobic gym 150 min/week in 3-5 sessions. None of them have no important changes in the diet in the last 12 months.

Exclusion criteria were: alcohol consumption, other known hepatic diseases (hemochromatosis, Wilson diseases, α_1-antitripsin deficiency), viral chronic hepatitis (markers for B and C hepatic viruses), treatment that can induce steatosis (such as Amiodarona, corticotherapy). Data of the patients were collected through a structured form which included: personal data ; medical history (hepatic diseases antecedents, diabetes mellitus, high blood pressure, dyslipidemia, smoking, alcohol consumption); clinical examination; biological explorations; transabdominal ultrasound; histological examination (the presence and degree of steatosis, of inflammation, of hepatocitary balonisation)**;** non invasive tests for simple steatosis and for NASH (inflammation). For the alcohol consumption patients completed a questionnaire based on the Behavioral Risk Factor Surveillance System 2006, writing down the number of consumed alcohol glasses per day and week and the type of consumed alcohol. We considered minimum alcohol consumption a quantity below 20 g pure alcohol/day for women and below 30 g pure alcohol/day for men. For the level of physical activity we used the Global Physical Activity Questionnaire (GPAQ) developed by WHO for physical activity surveillance and collecting information on physical activity participation in three , as well as sedentary behaviour.

The diagnostic of metabolic syndrome was accepted if at least three of the following five criteria have been present: abdominal circumference (AC) over 80 cm in women and over 94 com in men; serum triglycerides ≥150mg/dl (1.7 mmol/L) or hipolipemiant treatment; HDL cholesterol < 40mg/dl (1mmol/L) in men, < 50mg/dl (1.3mmol/L) in women or treatment for HDL-cholesterol; blood pressure ≥ 130/85 mmHg or anti high blood pressure treatment; glycaemia a jeune ≥100mg% or anti diabetes treatment for hyperglycaemia.

Calculation and interpretation the body mass index (BMI) was done by measuring the weight and the height of the patients, and we used the following formula: BMI = present weight (Kg/m^2)/height^2(m^2).The interpretation of the results was made as follows: normal weight BMI = 18.5-24.9$Kg/m^{2;}$ overweight BMI = 25-29.9Kg/m^2 ; 1st degree obesity BMI = 30-34.9Kg/m^2 ; 2nd degree obesity BMI = 35-39Kg/m^2 ; 3rd degree obesity BMI ≥ 40Kg/m^2 .

The abdominal circumference (AC) was measured at half the distance between the superior iliac crest and the costal margin, at the level of the navel, on median axillary line. After measuring the hip circumference we calculated the report of the two circumferences to determine the type of distribution of the adipose tissue: AC/HC ≥0.85 for women and ≥0.9 for men represented abdominal type obesity (android obesity) [19,20].

Blood samples were collected à jeune, from vein blood, after 12 hours of fasting.

Based on the clinical and laboratory data we calculated the following non-invasive scores: for steatosis FLI (fatty liver index) and NAFLD liver fat score and for steatohepatitis the scores published by Pierre Gholam in 2007 and Fernanda Pulzi in 2011(21,22,23).

FLI was calculated based on the following formula:FLI= $e^L/(1+e^L)*100$, where $L=0.953*\log_e$(triglycerides) +0.139*IMC +0.718*$\log_e(\gamma GT)$ +0.053*(AC) -15.745. A value below 30 excludes the hepatic steatosis, and the values ≥60 confirm the presence of the hepatosteatosis.

For the NAFLD fatty liver score= -2.889 +1.179*SM (yes=1/ bo=0) +0.454*DM type 2 (yes=2/no=0) +0.145*the insulinemia +0.038*GOT -0.936*(GOT/GPT) [185]. A value greater than -0.640 indicates the presence of the steatosis.

In the statistic model of Gholam (2.627*ln GOT +2.13 for the cases with diabetes), a value over 8.22 is predictive for NASH.

The other score for the steatohepatitis is calculated by granting 1 point for each of the following modifications: ALT≥30U/L, GGT≥30U/L, AST/ALT ≤1 and ultrasound aspect of hepatic steatosis. A score ≥4 is in favour of the NASH diagnosis. (24,25,26,27)

For the histopathological examination, the hepatic tissue fragments have been fixed in formalin (10%), then included in paraffin, sectioned in microtome and processed in the following staining: haematoxylin-eosin (HE), van Gieson, Gomori, Masson's trichrome.(28,29). NAFLD is characterised as defining elements by steatosis, namely the presence of fat representing 5% of the weight of the liver or in optical microscopy the presence of lipids in minimum 5% of the hepatocytes. The disposition of the fat is predominantly macrovesicular, observing in the hepatocyte a single drop of lipids which pushes the nucleus towards the periphery. The balonised hepatocytes denote a cellular aggression, they appear to be enlarged, swollen with or without Mallory bodies in cytoplasm. The lobular inflammation is usually moderated, the inflammatory infiltrate is mixed (with PMN and monocytes), and in some cases the portal inflammation can be present too, however without special characters. The characteristic pattern of the fibrosis in NASH is represented by the initial deposit of the extracellular matrix in area 3, perisinusoidal. In evolution can occur the periportal fibrosis with the formation of fibrous septa, subsequently fibrosis in bridges and cirrhosis (30,31).

The statistic analysis: The measured parameters for the subjects included in this study were stocked in Excel files, and for the data processing one used the Microsoft Excel (Microsoft Corp., Redmond, WA, USA) program, with the XLSTAT suit for MS Excel (Addinsoft SARL, Paris, France). All the continual variables were reported as average ± standard deviation (SD). For the comparison of the non invasive scores with the results of the histological examination one used the Chi square test and Kendall's test. The p value<0.05 was considered statistically significant.

Results

During the performance of our research 50 patients with lesions of hepatic steatosis, having histological confirmation, were enrolled and

they were subsequently investigated. Of the 50 patients 18 were men and 32 were women, with an average age of 36.4 years with limits within 20 and 45 years.

After the calculation of the body mass index, 28% of the patients were overweight, 62% had a certain degree of obesity and only 5 patients (10%) had a normal weight. 52% suffered from diabetes mellitus and 90% met the diagnosis criteria for the metabolic syndrome.

From the histological point of view, the studied cases presented steatosis in proportion of 96%. 2 cases presented steatosis in a percentage below 5% and were classified as S0 (table 1).

The type of steatosis which predominated in this study was macrovesicular, with a single drop, having as result the nuclear eccentricity, because it occupied all the cytoplasm of the cells and rarely with more and smaller drops, but well defined in the cytoplasm (fig 1). We noticed a predominance of lesions in area 3.

Steatosis degree	**No. of cases**
S0	2
S1	19
S2	17
S3	12

Table 1- Steatosis degree.

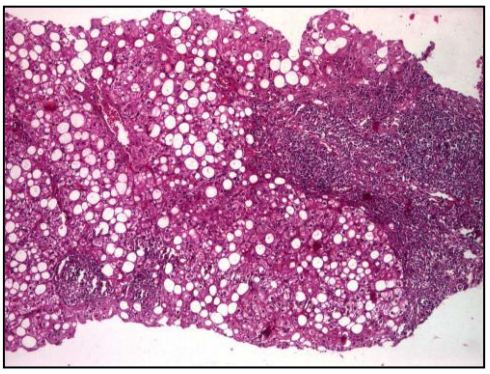

Figure 1. Macrovesicular hepatic steatosis, HE 40x

By comparing the anthropometric data and the results of the biochemical determinations in the patients with steatosis, one shall observe that there is no statistically significant correlation between these values and the steatosis degree.(27,28).

Table 2. The clinical and biochemical characteristics of the patients depending on the steatosis degree

	Steatosis S0 (n=2)	**Steatosis S1** (n=19)	**Steatosis S2** (n=17)	**Steatosis S3** (n=12)
Gender (men/women)	0/2	8/11	6/11	4/8
Age (years)	31±5.65	32.78±12.58	40.17±4.42	37.66±7.79
Diabetes mellitus (absent/present)	2/0	12/7	7/10	3/9
Metabolic syndrome (absent/present)	2/0	1/18	2/15	0/12
Abdominal circumference (cm)	84.5±2.12	103.36±7.46	106.29±13.2	104.08±10.1
Abdominal circumference/hip circumference	0.85±0.028	0.96±0.098	0.94±0.079	0.93±0.07
BMI (kg/m^2)	24.5±0.42	29.5±2.27	31.84±5.4	31.03±4.07
Normal weight (patients number)	2	0	2	1

Overweight (patients number)	0	9	2	3
1st degree obesity (patients number)	0	10	9	6
2nd degree obesity (patients number)	0	0	3	2
3rd degree obesity (patients number)	0	0	1	0
Total cholesterol (mg/dL)	179.5±5586	203.31±45.19	195.76±42.36	219.41±29.26
HDL-cholesterol (mg/dL)	74±24	49.6±17	42.1±11.6	41.9±8.7
Triglycerides (mg/dL)	63±25	178±58	189±118	201±50
Triglycerides /HDL-cholesterol	0.84±0.07	4.02±1.84	5.17±4.47	5.09±1.95
GOT (UI/L)	28±4.24	36.52±12.4	45.05±18.7	58.25±32.5
GPT (UI/L)	28±	44.68±28.3	49.76±23.7	88.33±42.1
GOT/GPT	1±0.15	0.92±0.26	0.98±0.37	0.66±0.16
GGT (UI/L)	12.5±6.36	91.05±127.67	48.88±26.44	68.33±41.09
Albumin	4±.28	4.23±0.33	4.04±0.29	4.21±0.39
Fatty liver index	10.13±1.68	78.39±14.21	79.36±23.5	85.65±14.89
NAFLD liver fat score	-1.81±0.18	1.88±2.71	2.26±1.90	9.3±11.82

Based on the clinical and biochemical data we calculated two scores of prediction of the fat hepatic load: Fatty liver index (FLI) and NAFLD liver fat score (NAFLD-LFS). In 92% of the patients with steatosis degree ≥ S1 (n=48) the results of the scores were over the values considered predictive for the hepatic steatosis, the correlation with the histopatholgical examination being high statistically significant (p Chi square<0.0001, p Ficher=0.0049) (table 2 and 3 and figures 2).
The balonisation of the hepatocytes, a characteristic which expresses the cellular lesion, was found in 22 of the cases (44%). There was no statistically significant relation (p=0.497143) between the presence of the steatosis and of the balonisation (figure 2).

Table 3. The repartition of the non invasive scores depending on the presence of the steatosis

	Without steatosis	**With steatosis**	**Total**
FLI<30	100,00%	4,17%	8,00%
FLI>30	0,00%	95,83%	92,00%
NSFLD-LFS<-0.64	100,00%	4,17%	8,00%
NSFLD-LFS>-0.64	0,00%	95,83%	92,00%

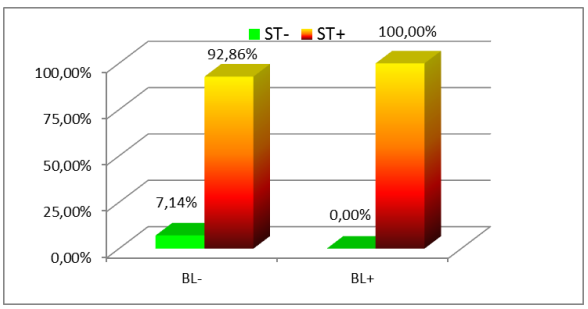

Figure 2. The relation between the presence of the balonisation and the presence of the steatosis

The affected cells have often been mixed in the steatosis areas. The hepatocitary balonisation was mostly observed in the perisinusoidal fibrosis regions detectable by HE (figure 3). The lobular

inflammation is part of the steatohepatitis diagnosis. Of the 50 patients, 18 presented steatohepatitis lesions, the lobular inflammation being mild in 72% of the cases and moderate in 28% of the cases (Table 4). There was no case with severe inflammation.

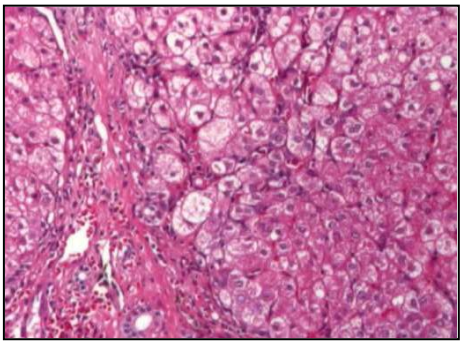

Figure 3. Hepatocitary balonisation, HE, 400x

Table 4. The degree of the inflammation

Lobular inflammation	Nr. of cases
0	32
1	13
2	5
3	0

The inflammatory infiltrate was predominantly chronic, with the presence of the monocytes, the PMNs being observed in 20% of the cases (figure 4). For the studied cases there was no correlation between the presence of the steatosis and of the inflammation, nor between the degree of the two types of lesions (figure 5).

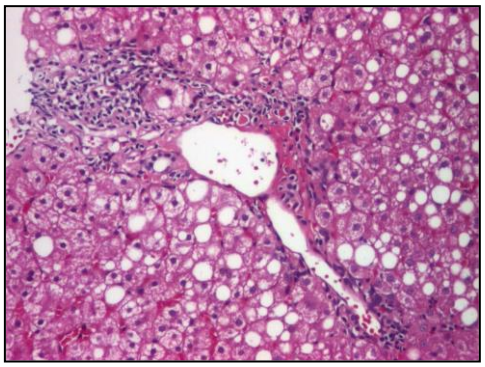

Figure 4. Mild necroinflammatory activity, HE 200x

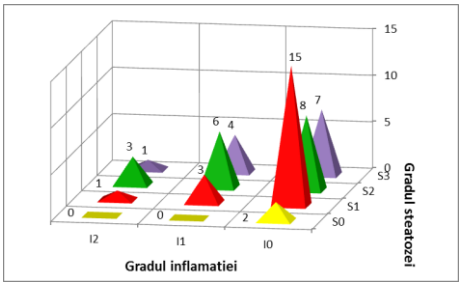

Figure 5. The relation between the degree of the inflammation and the degree of the steatosis

By calculating the NAS score, we obtained values contained between 0 and 6. For the 0, 1 and 2 scores, none of the cases fulfilled all the NASH criteria (steatosis, inflammation, balonisation).(29,30). For the 3 and 4 cases, of 15 cases, only 5 cases associate thee criteria. All the cases with NAS≥5 , are included in the defined NASH (table 5).
There was a statistically significant strong connection between the NAS score and the steatosis and inflammation degree (p<0.0001) (Table 6).

Table 5. The NASH definition by means of the NAS score

NAS score	Nr. of cases
0	2
1	14
2	9
3	7
4	8
5	4
6	6
7	0
8	0

Table 6. The relation between the NAS score and the degree of steatosis and inflammation

Statistic parameters	NAS-steatosis Correlation	NAS-inflammation Correlation
p Chi square	< 0.0001	0.00013
Cramer's V	0.786	0.620
Your Kendall	0.698	0.646
p Kendall	< 0.0001	< 0.0001

For the patients in the lot we calculated two scores quoted in literature as predictive for NASH: The Gholam score with a reference value of 8.22 and the score for hepatitis conceived in 2011 whose value ≤ 2 helps for the exclusion of the patients with NASH. (17,21,31). In all the patients in the lot, the values of the Gholam score was over 8.22, so that they could not be compared to the results of the histpathological results. As for the score for hepatitis, its was significantly correlated to the histological lesions of NASH (p Chi square=0.027) (Figure 6)

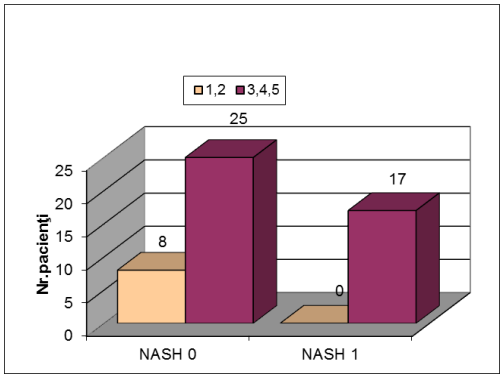

Figure 6. Correlation between hepatitis score and histological lesions.

Discussions

The liver represent an important organ in systemic metabolism, with substantial contribution to the development of insulin resistance and type 2 diabetes mellitus. NAFLD has a high prevalence in diabetic patients.

The liver, adipose tissue and skeletal muscle are the most important target tissue for insulin. In the liver, insulin regulates glucose metabolism, while in the adipose tissue insulin reduces the hormons sensitive lipase activity leading to the prevention of free fatty acids output from adipocytes. In adipose tissue, insulin allows the

esterification of free fatty acids and the storage of triglycerides. When insulin resistance occurs, free fatty acids are stored in non-fat tissues such as the liver, their accumulation leading to lipotoxicity. So, most of the obese patients have NAFLD and type 2 diabetes mellitus. It is well known that the first treatment for these patients is weight loss with diet and physical exercises. But, as we observed in our study , physical activity, without diet, couldn't lead to weight loss or to improve hepatic steatosis. For this reason, it is necessary to have some validate mathematical scores instead of liver biopsy.

Although the hepatic biopsy together with the histopathological examination is considered the golden standard in diagnose the stage of NAFLD, the criteria for the evaluation of steatosis are not very well established. The generally accepted value for the hepatic steatosis, based on the measurement of the lipid content, is 5%, but in the literature the value considered necessary for a diagnosis of steatosis varies from "any quantity" to 15-30%. Another point of view, based on modern imagistic techniques (spectroscopic magnetic resonance) indicates a value ≥5.56 for the diagnosis of the steatosis. In the lot of 50 patients with biopsy performed intra-operator there were two cases of steatosis under 5% which were classified as S0 (absence of the steatosis).(15,24,25).

In our study the non invasive tests used showed a statistically significant correlation with the steatosis on the hepatic biopsies. In relation to the steatohepatitis one was not able to interpret the Gholam score due to the fact that all the values were situated above the threshold value of 8.22 no matter the histological presence of NASH. The difference from the literature could be explained by the population with morbid obesity BMI> 40 Kg/m^2 in the original study , while in the present study the average value of the body mass index was 30.46 Kg/m^2.

Regarding physical activity, according the Global Physical Activity Questionnaire (GPAQ) we evaluated physical activity participation in three domains: activity at work, travel to and from places and recreational activities. For the calculation of a categorical indicator, the total time spent in physical activity during a typical week and the intensity of the physical activity were taken into account. WHO reccomandations showed that throughout a week, including activity for work, during transport and leisure time, adults should do at least 150 minutes of moderate- intensity physical activity OR 75 minutes of vigorous-intensity physical activity OR an equivalent combination of moderate- and vigorous-intensity physical activity achieving at least 600 MET-minutes.In our study the percentage of respondents not meeting WHO recommendations on physical activity for health (respondents doing less than 150 minutes of moderate intensity physical activity per week, or equivalent was of 100%.

Conclusions

- The distribution on genders of the patients proved a greater incidence of the disease in women than in men. The histological lesions of steatohepatitis were in a greater proportion still in women.
- The performance of hepatic biopsy in patients, allowed more accurate establishment of the role of non-invasive tests in the diagnosis of the lesions of steatosis, steatohepatitis and hepatic fibrosis. Although the serologic determinations for the calculation of these scores are accessible on a large scale, the non-invasive tests are most useful for the exclusion of the evolution lesions and for confirmation of the advanced stages of the disease.
- The level of the transaminases did not reflected the severity of the hepatic disease appreciated through biopsy, so we cann't use them as a marker of NAFLD progression.
- Physical activity, in absence of a correct diet, is not enough for an improvement of the patient condition.
- Furthermore, interdisciplinary studies are needed to examine the individual contributions of physical inactivity and overnutrition to the escalating prevalence of NAFLD.

Acknowledgment

Ethics statement

This study was performed in accordance with the principles of Helsinki Declaration and Good Clinical Practice and was approved by Ethics Committee of Emergency County Hospital and Centre of Research in Gastroenterology and Hepatology Craiova. All patients provided written informed consent.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All authors had an equal contribution to the manuscript .

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