

Skin autofluorescence is high in patients with cirrhosis – Further arguing for the implication of Advanced Glycation End Products

To the Editor:

Guimaraes et al. recently reported that the Advanced Glycation End products (AGE) induces the production of reactive oxygen species in hepatic stellate cells, providing an attractive mechanism for the development of liver fibrosis in the metabolic syndrome [1]. As mentioned by the authors, the deleterious consequences of the accumulation of AGEs are well documented in the context of diabetes [2] or chronic renal failure [3], whereas they have been less reported in cirrhotic patients. High levels of N^ε-(carboxymethyl)lysine “CML” [4] or other fluorescent AGEs [5] have been detected in the serum of these patients using ELISA or liquid chromatography–mass spectrometry assays [6], but these analytical methods are not routinely available, and the accumulation in other tissues, requiring biopsies, has not been reported. The optic properties of most AGEs may in fact help to provide further evidence for this accumulation.

The fluorescence of AGEs, as used by Guimaraes et al. to confirm the glycation of AGE-BSA that they applied to hepatic cells [1], allows for assessment of the accumulation of AGEs in the skin, in a simple and non-invasive manner, by measuring skin autofluorescence (AF) with the AGE-Reader (DiagnOptics BV, Groningen, The Netherlands). Briefly, the AGE-Reader illuminates approximately 1 cm² of skin (which is guarded against surrounding light) with an excitation light source of 300–420 nm (peak excitation ~350 nm). AF is calculated by dividing the average light intensity emitted per nm over the 420- to 600-nm range by the average light intensity emitted per nm over the 300- to 420-nm range. The value of this technique is supported by correlations between skin autofluorescence and collagen-linked fluorescence (CLF; excitation at 370 nm, emission at 440 nm) following pepsin digestion, and the pentosidine (by HPLC), N^ε-(carboxyethyl)lysine (CEL) and N^ε-(carboxymethyl)lysine (CML) (by gas chromatography and mass spectrometry) concentrations in skin biopsies, ($r = 0.47$ – 0.62 , $p < 0.002$) [7], and it is a strong predictor of cardiac mortality in patients with type 2 diabetes

[8]. We are not aware of any report about skin AF in patients with cirrhosis. The potential influence of an icterus, that may modify the optic properties of the skin, is also unknown.

Using the AGE-Reader, we have measured the forearm skin AF of 32 patients with cirrhosis (diagnosis on liver biopsy; alcoholic, viral, and metabolic origins), 16 patients with type 2 diabetes and at least one previous cardiovascular event, and seven healthy control subjects who were members of our staff (physicians, nurses). All participants signed an informed consent. Among the cirrhotic patients, 13 had jaundice icterus which was confirmed by the dosage of bilirubinemia. The results are presented as mean \pm SD, compared by ANOVA with a Bonferroni correction.

As expected, the 16 patients with diabetes had higher skin AF results than control subjects (3.4 ± 0.7 ; $p < 0.001$), but the skin AF were also higher in the patients with cirrhosis as compared with the control subjects (2.4 ± 0.6 vs 1.9 ± 0.2 ; $p < 0.05$). As shown in Table 1, the icteric patients had lower results than those without icterus ($p = 0.08$ vs cirrhosis without icterus). We, therefore, measured the AF of six normal subjects before and after painting their forearm with bilirubin (Calbiochem, La Jolla, CA 92037): this immediately reduced the AF (before: 1.7 ± 0.2 , after painting with bilirubin: 1.4 ± 0.2 ; $p < 0.05$).

Although these results are preliminary, the higher AF, as we found, further argues for the implication of AGEs in liver fibrosis. Of course, such clinical data do not replace mechanistic studies: as mentioned by Guimaraes et al., the liver is critical for the clearance of AGEs [9], so their accumulation in the tissues of patients with cirrhosis may in fact be a consequence, rather than a cause, of liver damage. Even in this case, deleterious effects of this accumulation may occur outside the liver: further vascular complications have been reported in patients with type 2 diabetes and Non alcoholic Fatty Liver Disease (NAFLD) [10]. In any case, we feel that the hepatologists should be informed that the accumulation of AGEs in tissues can be evaluated by non-invasive techniques, as we did, and that cirrhosis and icterus influence the results.

Table 1. AutoFluorescence results in patients with cirrhosis, type 2 diabetes and control subjects.

	Control subjects	Cirrhosis with icterus	Cirrhosis without icterus	T2D	<i>p</i>
n	7	13	19	16	
Age	48 \pm 6	56 \pm 10	56 \pm 10	59 \pm 8	n.s.
Glycemia (mg/dl)	-	114 \pm 13	109 \pm 40	190 \pm 62	<0.001
Bilirubinemia (mmol/L)	-	159 \pm 138	27 \pm 9	-	<0.001
AF	1.9 \pm 0.2	2.2 \pm 0.6	2.7 \pm 0.7	3.4 \pm 0.7	<0.001

Letter to the Editor

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