Letter to the Editor KI-07-20-1331 15 July 2020

"Green/apple-green birefringence": unfit for purpose?

Like most people working on amyloid, Colombat et al.¹ report that Congo red-stained amyloid shows "green birefringence" or "apple-green birefringence", although their figures (5b, 6b, 7b, and 7d-f) show various colors, and in at least two (7e and 7f), green is difficult to see. We wrote to Kidney International in 2012 to point out a similar discrepancy between so-called "apple-green birefringence" and multiple colors in an image².

The Nomenclature Committee of the International Society of Amyloidosis gave up "green birefringence" in 2014, preferring "green, yellow or orange birefringence", although "yellow-green birefringence" appeared in 2018³. When even this prestigious body is uncertain what colors are seen, there is little surprise that most workers stick automatically with "green (or apple-green) birefringence", despite its demonstrable unsuitability. Why are other colors not mentioned? Is it really amyloid if no green is seen?

How "green (or apple-green) birefringence" became firmly established is a story of ignorance and misunderstanding of the physical optics of Congo red-stained amyloid, and of widespread acceptance of dogmatic assertions that are disproved by everyday experience⁴. We have explained how green is seen on its own only in perfect conditions of polarizing microscopy, how mixtures of colors are usually seen, which could cover the spectrum from blue to orange, may not include green, could include white or red, and can change, and how the best expression is to say that Congo red-stained amyloid between polarizer and analyzer shows anomalous colors, meaning colors different from the red of Congo red in ordinary illumination^{2,4}.

1. Colombat M, Aldigier J-C, Rothschild P-R, et al. New clinical forms of hereditary apoA-1 amyloidosis entail both glomerular and retinal amyloidosis. *Kidney Int* 2020; **98**: 195-208.

Howie AJ, Owen-Casey MP. "Apple-green birefringence" of amyloid stained by Congo red.
Kidney Int 2012; 82: 114.

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3. Howie AJ. The nomenclature committee of the international society of amyloidosis: back towards "green birefringence". *Amyloid* 2019; **26**: 96.

4. Howie AJ. Origins of a pervasive, erroneous idea: the "green birefringence" of Congo redstained amyloid. *Int J Exp Path* 2019; **100**: 208-221.

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