2-Hydroxyglutarate is not a metabolite; D-2-hydroxyglutarate and L-2-hydroxyglutarate are!

In the recent study by Wang et al. (1), the prognostic significance of the assessment of serum 2-hydroxyglutarate (2-HG) concentrations in acute myeloid leukemia was investigated. The authors report that in 87% of the patients with high serum concentrations of 2-HG, mutations in isocitrate dehydrogenase (IDH)1/IDH2 occurred, versus 29% in the group with moderately high 2-HG concentrations. This finding led to their suggestion that other genetic or biochemical events cause 2-HG elevation.

Serum 2-HG carries an asymmetric carbon atom in its carbon backbone and therefore occurs in two distinct forms: L-2-hydroxyglutarate (L2HG) and D-2-hydroxyglutarate (D2HG). It is important to note that both D2HG and L2HG are normal endogenous metabolites found in all human body fluids.

Although L2HG and D2HG are identical in their physical properties, such as boiling point, solubility, and so forth, in the world of biochemistry these metabolites are entirely different entities. Routine analytical methods to detect 2-HG are not able to differentiate the measured signal into D2HG and L2HG, and as a consequence the sum of the two metabolites is measured.

The analytical procedure to measure 2-HG performed by Wang et al. (1) is not able to differentiate between D2HG and L2HG. The gain-of-function mutations in IDH1/2 enzymes result in genesis of neomorph enzymes that produce vast amounts solely of D2HG, but these neomorph enzymes do not produce L2HG. The measurements of serum 2-HG in the report by Wang et al. (1) is not corrected for the endogenous serum levels of L2HG, which in healthy individuals is equal to or exceeds the level of D2HG. As a consequence, minor increases in the serum levels of D2HG might be missed and increases of L2HG might yield false-positive results.

In the original report by Dang et al. (2), only the use of an analytical method that differentiates between D2HG and L2HG [i.e., chiral derivatization followed by LC-MS/MS (3)] unraveled that the *IDH* mutant enzyme produces D2HG solely. It is of the utmost importance that the scientific community is aware that routine analytical techniques measure total 2-HG (sum of D2HG and L2HG), and that in fact 2-HG as such does not exist.

In conclusion, the detection of D2HG as biomarker related to *IDH* mutations should be performed by those methods able to quantify D2HG separate from L2HG. Furthermore, authors should clearly state how the metabolites have been measured and should use the term "total 2-HG" to describe results from measurements generated by a nondifferential analytical method.

Eduard A. Struys[1]
Metabolic Laboratory, Clinical Chemistry, Vrije Universiteit Medical Center, Amsterdam 1081HV, The Netherlands

1 Wang J-H, et al. (2013) Prognostic significance of 2-hydroxyglutarate levels in acute myeloid leukemia in China. *Proc Natl Acad Sci USA* 110(42):17017–17022.
2 Dang L, et al. (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462(7274):739–744.
3 Struys EA, Jansen EE, Verhoeven NM, Jakobs C (2004) Measurement of urinary D- and L-2-hydroxyglutarate enantiomers by stable-isotope-dilution liquid chromatography-tandem mass spectrometry after derivatization with diacetyl-L-tartaric anhydride. *Clin Chem* 50(8):1391–1395.

Author contributions: E.A.S. wrote the paper.
The author declares no conflict of interest.

[1]E-mail: E.Struys@vumc.nl.