Editorial

Simona Ferraro*, Giacomo Biganzoli, Valeria Calcaterra, Gianvincenzo Zuccotti, Elia Mario Biganzoli and Mario Plebani

The relevance of establishing method-dependent decision thresholds of serum folate in pregnancy and lactation: when the laboratory stewardship meets the health-care needs

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The characterization of folate status in women in pregnancy and lactation, at risk of deficiency due to altered vitamin homeostasis and increased request, is crucial to endorse preventive intervention health policies [1]. Several authors have recently emphasized that, as coenzyme, folate is involved in various biological process (i.e. DNA, purine, ammino acids and lipid synthesis, placental amino acid transportation) that are critical for normal fetal growth and development, reinforcing the recommendations on folic acid (FA) supplementation in pregnant women, which has been shown to reduce several congenital anomalies (neural tube and heart defects, urinary tract anomalies, oral facial clefts, and limb defects) [2]. The supplementation is further to be considered mandatory in pregnant women exposed to different risk factors, such as genetic, environmental and dietary, including those who are taking medications known to have interactions with folate metabolism/absorption, all leading to low maternal serum folate levels [2]. Anyway, the current clinical practice guidelines (CPGs) endorse FA supplementation discarding the use of its determination in serum to assess the risk of deficiency and/or its concentration at baseline. Poor confidence on the diagnostic accuracy of serum folate assays persists in current CPGs although recent standardization efforts have greatly improved inter-method variability and precision [1]. Anyhow, the poor interchangeability of serum folate results obtained from different assays should be critically appraised by CPGs and a recommendation against the use of harmonized thresholds to address the need of FA supplementation should be released. We have recently reported the method-dependent cut off points to characterize folate deficiency and individualize the need of FA supplementation in pregnancy (table) recommending however to pursue a cost-effective use of the test [2]. Accordingly, folate retesting in the case of recently interrupted or during supplementation is useless since endogenous physiological folate generally consists of a 3 months' worth supply [1]. In pregnancy and lactation, a serum folate retesting at 3–6 months should be endorsed in order to monitor the actual correction of the deficiency and the maintenance of vitamin homeostasis [1]. More recently, some authors have further emphasized the crucial relevance to optimize folate and vitamin B12 circulating levels in pregnant women through vitamin supplementation according to an individualized approach, by adding new epidemiological evidence [3]. It has been highlighted that the interaction between highest and lowest levels of serum folate and vitamin B12 respectively, being a common finding in these subjects, was associated to an increased risk of gestational diabetes [3]. For instance, the evidence by Saravanan et al. is challenging and essential to definitely confirm and translate into this clinical framework the association between the risk of diabetes and the vitamins' status observed using *in vitro*/*in vivo* models [3–6]. Indeed,

Mario Biganzoli and Mario Plebani contributed equally to this work.

***Corresponding author: Simona Ferraro,** Endocrinology Laboratory Unit, ASST Fatebenefratelli-Sacco, Ospedale 'Luigi Sacco', Via GB Grassi 74, Milan, Italy, E-mail: simona.ferraro@asst-fbf-sacco.it
Giacomo Biganzoli and Elia Mario Biganzoli, Medical Statistics Unit, Department of Biomedical and Clinical Sciences L. Sacco, "Luigi Sacco" University Hospital, Università degli Studi di Milano, Milan, Italy
Valeria Calcaterra, Pediatric and Adolescent Unit, Department of Internal Medicine, University of Pavia, Pavia, Italy; and Pediatric Department "V. Buzzi", Children's Hospital, Milan, Italy. https://orcid.org/0000-0002-2137-5974
Gianvincenzo Zuccotti, Pediatric Department "V. Buzzi", Children's Hospital, Milan, Italy; and Department of Biomedical and Clinical Science, University of Milan, Milan, Italy
Mario Plebani, Department of Medicine -DIMED, University of Padova, Padua, Italy. https://orcid.org/0000-0002-0270-1711

as coenzymes, folate and B12 are involved in various biological pathways (i.e., aforementioned for the folate, whereas B12 mainly promotes fatty acids/amino acids degradation) whose balance is warranted by the maintenance of adequate levels of both vitamins in order to assure the normal physiological functions [6]. Undoubtedly, folate and particularly for its depletion, is the most investigated vitamin in the framework of gestational risk and diabetes [4–7]. *In vitro* models of the pathophysiology of diabetes have shown that the folate deficiency is associated with the apoptosis of islet β cells [4]. Noteworthy, the excessive exposure to FA may promote DNA methylation patterns thus contributing to trigger the inflammatory response, well characterized, although differentiated, in diabetes type I and II [5]. The novel results, about the "U-shaped" relationship between fasting serum folate and plasma glucose levels, represent robust clinical evidence on the need to revise current CPGs which recommend supplementation without requiring serum folate testing at baseline [1–3]. This requirement is further reinforced by recent pharmacovigilance data, revealing that the rate of patients experiencing adverse effects ascribed to FA supplementation is considerable, mostly associated to an overdose (i.e. above the recommended UL of 1 mg/day) and/or to an excessive exposure, the latter being a common finding in pregnancy as prenatal supplements may contain 1,000 µg FA [7]. Therefore, a close monitoring of FA exposure in gestation should be endorsed, accounting that: (a) highest circulating folate levels may further increase the risk of hypertension and insulin-resistance in pregnant women and exert potential adverse effects on offspring [8]; (b) an early and unpredictable folate decay may occur due to an increased requirement for the vitamin [1]. Consequently, in pregnancy and lactation, a recommendation on the use of serum folate testing before supplementation and 3–6 months after its discontinuation should be further endorsed and included in current CPGs to check the actual maintenance of vitamin homeostasis and to avoid adverse effects associated to vitamin deficiency or overload [1].

Anyway, the definition of reference ranges (RR) of serum folate, related to pregnancy, is crucial to pragmatically fulfill the optimization of circulating folate levels and to maximize the benefit-risk ratio of folic acid supplementation using an individualized approach [1]. The assessment of appropriate RR in this population first involves the identification of reliable lower and upper reference serum folate levels (LRL/URL) from studies focused on clinical outcomes [1–3], considering that exceeding these threshold values likely increases the risk of adverse outcomes in pregnant women and/or in the offspring [4]. Second, the LRL and URL require to be "calibrated" to commercially available assays since the poor harmonization of the serum folate results obtained by the different methods [1]. In other words, the LRL/URL retrieved by each study, reporting the immunoassay used, should be converted into the corresponding concentrations detected by other commercially available immunoassays, by exploiting the equations determined from head to head comparisons [1, 2]. Considering the definition of the URL, we have to account that recent clinical evidence provides robust data on the quantitation of "circulating folate excess" [3]. Thus, the value of the third tertile of the folate serum levels distribution, that is 52.7 nmol/L (i.e. 23.3 µg/L) may be confidently assumed as URL.

Since this determination has been performed on the Roche Cobas analytical platform, in Table 1 we provide the conversion of this URL into concentrations estimated by other commercially available assays to generalize the use of the URL to other assays used in the clinical practice. This is relevant, as a clinically validated URL using an appropriate robust statistical approach (i.e. cubic splines) able to capture the U-shaped vitamin relationships with metabolic markers in this population of subjects has never been provided. In fact, the folate excess is generally assumed at a threshold of 45 nmol/L (i.e. ~20 µg/L), simply because this value represents the upper limit of the calibration curve for most of commercially available assays, although the same methods may characterize concentrations up to 40 µg/L after applying the recommended dilution [1]. As observed by Saravanan et al. this is because most of the studies use only the estimated folate intake to assess the association between this vitamin and outcomes in pregnancy, and this is also the reason why we are unable to define the administered daily FA dose triggering the increase of serum folate concentrations up to 20 µg/L [1, 3].

Considering the definition of the LRL, a serum folate concentration between 8.5 and 10.0 µg/L, depending on the assay used (Table 1), should be considered the lowest value which may be tolerated to exclude the onset of adverse effects in pregnancy and anomalies in the offspring due to folate supply depletion [3]. In conclusion, recent studies provide clinical robust evidence to carry out the definition of the RR of serum folate levels, that should be considered during pregnancy to individualize FA supplementation and monitor a possible discontinuation in order to maximize the benefits of the vitamin. The RR reported in the table and calibrated to the current immunoassays should be pragmatically included in the CPGs to effectively interpret folate results as an aid to optimize FA supplementation [1]. Our approach to the management of folate testing and results, with the aim to pursue cost-effective health-care policy fully fulfills the novel pragmatic definition of laboratory

Table 1: Maternal folate lower and upper reference levels (LRL, URL) estimated according to different commercially available assays.

Roche Diagnostics Cobas e801 (folate III) µg/L (95% CI)	Abbott Diagnostics Alinity i µg/L (95% CI)	Beckman Coulter Dxl Access µg/L (95% CI)	Advia Centaur Siemens Healthcare Diagnostics µg/L	Maternal folate serum Rl to prevent adverse maternal/fetal outcomes
8.7[a]	8.50 (8.12, 8.94)	9.63 (9.38, 9.93)	8.70 (8.20–9.14)	LRL established according to the evidence that lower serum folate concentrations were associated to the risk of neural tube defects in pregnancy [1, 2]
23.3[a]	21.65 (20.32, 22.92)	24.23 (23.21, 24.93)	21.65 (20.26, 22.98)	URL established according to the evidence that higher concentrations were associated to the risk of gestational diabetes [3]

[a]Originally expressed as nmol/L: 1 ng/mL=2.265 nmol/L.

medicine, that is "translating results into actionable information for improving the care and/or maintaining the wellness of both a single individual and an entire population" [9]. Under this perspective, laboratory professionals have engaged several efforts to enhance their skills, create a clinical-laboratory interface and include in laboratory reports assistive information which can support the design of personalized treatment plan [10]. Undoubtedly FA supplementation has changed the epidemiology of several congenital anomalies, but pharmacovigilance evidence and cost-effective goals mandatorily imply an appropriate use of laboratory results in face of the poor harmonization of the methods [1]. Although folate has been recently considered an "ancillary marker" in those countries fulfilling fortification policies, for the aforementioned reasons it represents a good example of how laboratory professional skills may enter in a broader vision of health care and patient's needs [11].

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