Clinical validation of a mass spectrometer-based method for measuring nicotine-metabolite ratio to optimize smoking cessation pharmacotherapies

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A major cause of the high percentage of drug failures after smoking cessation pharmacotherapy relies on the inter-individual variability in nicotine metabolism (Benowitz *et al*., 2010). In our experience at the Smoking Cessation Centre of the University Hospital of Pisa, abstinence rate at 1 year is about 15%. Since there is no consensus on how to select the most appropriate drug for an individual smoker, it is important to identify strategies allowing clinicians to personalize smoking cessation pharmacotherapy. The ratio of two metabolites derived from nicotine smoking, 3'-hydroxycotinine (3HC) and cotinine (COT) (nicotine-metabolite ratio, NMR), reflects the activity of the liver enzyme CYP2A6 and it has been recently used to predict response to nicotine patch or varenicline (Lerman *et al*., 2015). To facilitate translation of study findings to health care professionals operating in the real life situation, we carried out a clinical validation of a mass spectrometer-based method for measuring the NMR in plasma samples obtained from 59 subjects attending the Smoking Cessation Centre of the University Hospital of Pisa. CYP2A6 genotyping was carried out to identify polymorphisms associated with enzyme deficiency. To define the nicotine metabolic phenotype we defined slow metabolizers as NMR<0.31 and normal metabolizers (including rapid metabolizers) as those with NMR ≥0.31. The NMR calculation was possible for 90% of participants while the remaining 10% were abstinent smokers with undetectable COT and/or 3HC plasma levels. One third of participants were slow metabolizers, whereas others were normal/rapid metabolizers. We demonstrated the presence of two specific variant alleles in the CYP2A6 locus associated with decreased nicotine metabolism. Specifically, 2 and 10% of study subjects were heterozygous for CYP2A6*2 and *9 variant alleles, respectively. According with phenotype assessment, CYP2A6 heterozygous mutants with decreased activity allele had NMR values <0.31. No differences were observed for NMR when sample preparation was performed with SPE cartridge (Waters Oasis MCX 1 ml, 30 mg) or Phree (Phenomenex) 96 well plates (r^2: 0.920). Overall, these findings provide evidence that the NMR can be an accurate and robust biomarker in the real life setting. At present, preliminary analyses to test whether NMR in plasma and saliva also support the reliability of a saliva test for measuring the phenotype for nicotine metabolic status in active smokers.