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Original Article

A product of immunoreactive trypsinogen and pancreatitis-associated protein as second-tier strategy in cystic fibrosis newborn screening

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Abstract

Background: In cystic fibrosis newborn screening (CFNBS), immunoreactive trypsinogen (IRT) and pancreatitis-associated protein (PAP) can be used as screening parameters. We evaluated the IRT × PAP product as second-tier parameter in CFNBS in newborns with elevated IRT.
Methods: Data on 410,111 screened newborns including 78 patients with classical cystic fibrosis (CF) from two European centers were retrospectively analyzed by discrimination analysis to identify a screening protocol with optimal cutoffs. We also studied differences in PAP measurement methods and the association of IRT and PAP with age.
Results: PAP values differed systematically between fluorometric and photometric assays. The IRT × PAP product showed better discrimination for classical CF than PAP only as second-tier screening parameter ($p < 0.001$). In CF patients, IRT decreased while PAP values remained high over years. In newborns without CF, IRT decreased after birth over weeks while PAP increased within days.
Conclusions: The IRT × PAP product performs well as second-tier cutoff parameter for CFNBS. Screening quality parameters depend on the analytic method and on age at blood collection.

Keywords: Cystic fibrosis; Newborn screening; Immunoreactive trypsinogen; Pancreatitis-associated protein; Cutoff

Abbreviations: AUC, area under curve; CF, cystic fibrosis; CFNBS, cystic fibrosis newborn screening; CFSPID, CF screen-positive inconclusive diagnosis; CI, confidence interval; IRT, immunoreactive trypsinogen; MI, meconium ileus; PAP, pancreatitis-associated protein.

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1. Introduction

The clinical and economic benefits of early diagnosis of cystic fibrosis (CF) highlight the necessity for effective strategies for CF newborn screening (CFNBS) [1–3]. However, there is currently no ideal single analyte available for CFNBS from dried blood spots. Immunoreactive trypsinogen (IRT) has been used as a sensitive parameter for CFNBS since 1979 [4,5], although an increased IRT is not specific for CF [6].

The limited specificity of IRT may be partly mitigated by quantifying an additional biochemical marker, pancreatitis-associated protein (PAP), which is increased in inflammatory pancreatic processes [7,8]. PAP was introduced as a second parameter for CFNBS by Sarles et al., considering IRT and PAP cutoffs sequentially, yet independently of each other [9]. Several screening programs based on variations of this protocol reached sensitivities between 76% and 96% [10–13], comparable to sensitivities of IRT/DNA-based protocols with or without different third-tier screening steps [14–17]. The original commercially available diagnostic photometric PAP assay has been revised since then and is now based on two monoclonal antibodies, likely resulting in a different dynamic range and specificity of PAP quantification [10]. A second assay version, using time-resolved fluorometric measurement instead of photometry, has become available. These developments suggest that original cutoff values for PAP may need to be reconsidered.

In contrast to IRT/DNA protocols, a combination of IRT and PAP measurements may allow for a more specific selection of patients who require further diagnostic tests such as *CFTR* gene (cystic fibrosis transmembrane conductance regulator; MIM#602421) molecular genetic analysis and/or sweat testing. Consequently, detection of CF screen-positive inconclusive diagnoses (CFSPID), parental distress, and costs may be reduced [18].

We aimed to identify the optimal test combinations and cutoffs for predicting CF. We hypothesized that combining the actual values of IRT and PAP as a product may allow for a higher sensitivity and specificity rather than a step-wise evaluation of independent IRT and PAP cutoffs. Moreover, we hypothesized that IRT and PAP concentrations depend on age of the newborn at the time of blood sampling. Finally, we compared our cutoff model to other published recommendations.

2. Materials and methods

2.1. Study cohorts and routine screening protocols

This retrospective study included all newborns with a gestational age of at least 32 weeks who underwent sampling for CFNBS at or after 36 h after birth, mostly within 72 h postnatally. This study was conducted at two European CFNBS centers, Dresden and Heidelberg (both in Germany). The recommended blood collection time was 36 to 72 h after birth [19]. At the Heidelberg center, preterm newborns of less than 32 weeks were also included for part of the study period; among those were 121 with elevated IRT.

Both centers started CFNBS by IRT quantification using the AutoDELFIA neonatal IRT immunoassay (PerkinElmer, Turku, Finland) [11,12]. The IRT cutoff ranged within and between laboratories from 60 to 74 μg/l. IRT values above the 99th percentile after repeated measurement were included in Dresden. In Heidelberg, due to repeated IRT testing after a first elevation above the 99th percentile, only the remaining 0.66% highest IRT concentrations were included.

PAP quantification was performed using the ELISA MucoPAP kit (Dynabio, Marseille, France). Photometric measurements were done in Heidelberg, whereas fluorometric measurements were used in Dresden (MucoPAP with Europium labeling, PerkinElmer; since 2013, MucoPAP F, Dynabio, Marseille, France). PAP concentrations were standardized based on the corrected blood volume of 3 μl per 3.2 mm diameter dried blood spot punch instead of 5 μl, as initially suggested by the kit's producer [20]. If PAP measurement after IRT elevation was not possible from the primary dried blood spot, both IRT and PAP were measured from a new blood sample.

In Heidelberg, but not in Dresden, mutation analysis of the *CFTR* gene was carried out as a second diagnostic step after IRT elevation, in parallel to PAP measurements. At both centers, a positive CFNBS result was defined as elevated IRT and elevated PAP. In Dresden, the cutoff combination according to Sarles et al. [9] was used: for IRT ≥ 50 μg/l, PAP ≥ 3.0 μg/l was defined as positive; for IRT ≥ 100 μg/l, PAP ≥ 1.67 μg/l was defined as positive [11,12]. In Heidelberg, a single PAP cutoff of 1.67 μg/l was applied for all IRT values above the 99th percentile. Additionally, an IRT above the 99.9th percentile independent of PAP was defined as positive at both centers ("safety net strategy") [11]. CF diagnosis was defined as a chloride concentration ≥ 60 mmol/l in two independent sweat samples via pilocarpin iontophoresis [21,22] or detection of two *CFTR* gene mutations (Heidelberg). Newborns with *CFTR* gene mutations of varying clinical consequence according to *CFTR*2 database or intermediate sweat chloride were classified as CFSPID [23,24].

Each CFNBS regional study was approved by local ethics committees [11]. Informed consent was given by parents. Data were acquired from February 2008 to June 2014 in Dresden and from April 2008 to April 2014 in Heidelberg. All statistical tests were two-tailed and the type I error level was 0.05. Analyses were performed using Stata 12.1 (StataCorp LP, College Station, TX).

2.2. PAP measurement method comparison

To ensure comparability of PAP results between centers using different analytic techniques, photometric and fluorometric PAP measurements were compared. We used manufacturer-provided dried blood PAP standards and controls from the fluorometric MucoPAP F kit as well as venous dried blood spots, measured with both photometric and fluorometric test kits. Concentrations were compared using linear regression.