distinguishable. When measured by spectrophotometer, readings for all samples were higher than those for the 0 ng/L calibrator; OOM extracts provided signals that were well above those of the 400 ng/L calibrator.

In summary, simple aqueous extracts from small portions of 6 different OOM-associated tumors revealed very high FGF-23 concentrations as assessed by the calibrator assay; FGF-23 concentrations were also readily detectable by a modified rapid test that takes <30 min to complete. Like intraoperative parathyroid hormone assays *(5)*, this rapid assay could be performed in or near the operating room, especially because visual inspection of the test plate was sufficient to detect FGF-23 in all 6 tumors tested. The assay may furthermore help define, intraoperatively, the disease-free margins of tumors located in areas that are difficult to access surgically.

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More Studies on Outcomes Using Biochemical Diagnostic Tests Are Needed: Findings from the Danish Society of Clinical Biochemistry

To the Editor:

The results of biochemical tests often lead to diagnostic and therapeutic interventions, and the real value of a test can be assessed only by taking into account the subsequent health outcomes. The importance of outcomes studies, and the challenges in performing them, was reviewed by Bruns in 2001 *(1)*, who argued that this type of study should be performed more frequently, and that such studies should be used to determine whether new tests should be implemented in clinical practice.

To investigate the extent to which this recommendation has been realized, a working group on evidence-based clinical biochemistry established by the Danish Society of Clinical Biochemistry undertook a pilot study to record the number and type of reports of diagnostic biochemical outcome studies published from January 2005 to January 2006 in 4 medical journals: *Clinical Chemistry, Clinical Chemistry and Laboratory Medicine*, *Lancet* and the *New England Journal of Medicine*. To be included as an outcome study, the reported study had to be designed to investigate outcomes in relation to a clinical or an economical variable of a well-defined clinical application of a biochemical test.

To identify reports of outcome studies, 2 authors manually went through reports published in each of the journals within a 12-month period. Detailed information on original full-length reports considered diagnostic biochemical outcome studies was registered together with the total number of original articles. Technical Briefs, Letters, Short Communications, Editorials, and Reviews were not included. When there were discrepancies in report selection by the 2 authors scrutinizing the same journal issues, a consensus decision was made in the entire author group. Selected outcomes reports were classified as investigating (A) direct clinical mortality or morbidity; (B) other clinical variables such as length of hospital stay, readmission rate, or satisfaction with care; or (C) economic outcomes.

A total of 829 original articles were registered, of which only 7 studies (0.8%) were classified as diagnostic biochemical outcome studies (Table 1). Six (of 231) of these original articles were published in the *New England Journal*

Table 1. Diagnostic biochemical outcome studies.[a]

Study report	Study design	Biochemical test	Outcome[b]
Smith AD et al. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med 2005;352:2163–73.	Randomized controlled trial (N = 97), 12-month follow-up	Exhaled nitric oxide	Frequency of exacerbations of asthma and mean daily dose of inhaled corticosteroid (A+B)
Crowther CA et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477–86.	Randomized controlled trial (N = 1000)	Blood glucose	Perinatal complications and maternal health-related quality of life (A+B)
Tsao MS et al. Erlotinib in lung cancer: molecular and clinical predictors of outcomes. N Engl J Med 2005;353:133–44.	Randomized controlled trial (N = 731)	Expression and number of copies of epidermal growth factor receptor	Responsiveness to Erlotinib (A)
Stramer SL et al. West Nile Virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451–9.	Prospective screening of all blood donors for West Nile virus; before-after	West Nile Virus RNA	Elimination of carriers of the virus from the US Red Cross blood supplies and no infections amongst recipients in 2003 and 2004 (A)
Busch MP et al. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460–7.	Mass testing of donor blood by amplification testing of minipools and individual donations	West Nile Virus RNA	In high-prevalence regions individual testing is worthwhile (cost-effective) due to detecting low-level viremic donors preventing infection amongst recipients (A+C)
Malone FD et al. First-trimester or second-trimester screening, or both, for Down syndrome. N Engl J Med 2005;353:2001–11.	Prospective cohort (N = 38 167)	Several biochemical screening strategies	Capability of different strategies for screening in prenatal detection of Down syndrome (A)
Petersen JR et al. Association of transcutaneous bilirubin testing in hospital with decreased readmission rate for hyperbilirubinemia. Clin Chem 2005;51:540–4.	Retrospective before-after (N = 8974)	Transcutaneous bilirubin testing	Readmission rate (B)

[a] Study reports published during 2005 in *Clinical Chemistry*, *Clinical Chemistry and Laboratory Medicine*, *Lancet*, and *New England Journal of Medicine*.
[b] Outcomes classified as direct clinical outcome, i.e. mortality or morbidity (A); other clinical outcome, e.g., length of hospital stay, readmission rate, or satisfaction with care (B); and/or economy-related outcome (C).

of Medicine and 1 of 179 original articles in *Clinical Chemistry*. Three articles were classified as A, 2 as A+B, 1 as A+C, and 1 as B.

An outcome study addresses the question of whether use of the studied intervention (in this case a laboratory test) leads to an anticipated outcome *(1, 2)*. The randomized controlled trial is a powerful design strategy for such studies, avoiding many pitfalls that occur with other study designs *(2)*. Three of the outcome studies identified in this pilot study used a randomized controlled trial design that involved some variant of a test-treat-counsel policy to be compared with a policy not involving the testing element.

Before-and-after diagnostic assessment of clinical impact is another appropriate design for evaluating clinical outcomes of the use of laboratory tests *(3)*, as elegantly demonstrated in the study by Stramer et al. (Table 1). This study investigated the effect of prospective screening of all blood donors for West Nile virus, and the results indicated that carriers of the virus were eliminated from the US Red Cross blood supplies, and no infections were detected among recipients after the introduction of this screening program.

We identified good examples of diagnostic biochemical outcome studies, but the absolute number of these studies was disappointingly low, indicating insufficient documentation of the health outcomes produced by diagnostic biochemical analyses. This insufficiency is probably attributable to multifaceted causes. An important aspect is undoubtedly the complexity and high costs of outcome studies *(1, 4)*, because many steps lie between test findings and outcome. Research is needed that addresses

methodological issues concerning design and conduct of test-outcome studies. Comprehensive discussions of this complex and important area are available *(1–4)*, and in the textbook *Evidence-Based Laboratory Medicine* the chapter on assessment of outcomes is especially relevant *(5)*.

Our purpose is to highlight the importance of moving from diagnostic accuracy studies to evaluations of the effects of test results on clinical decision-making and subsequent health outcomes. In agreement with other investigators *(1–5)*, we support efforts to increasing the use of outcome studies to enhance the effectiveness of healthcare policy and decision-making.

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Electrospray Ionization Mass Spectrometric Analysis of the Globin Chains in Hemoglobin Heterozygotes Can Detect the Variants HbC, D, and E

To the Editor:

We would like to point out that 2 recent articles in this journal about human hemoglobin (Hb) analysis *(1, 2)* give the false impression that variant globin chains with <6 Da mass difference from normal cannot be detected in heterozygotes by electrospray ionization mass spectrometry (ESI-MS). Kleinert et al. *(1)* state: "Two important drawbacks of the MS methods should be mentioned. First, its insufficient resolution prevents the detection of Hb mutations with small mass differences of the globin chains. The precision of normal low-resolution mass measurements was insufficient to distinguish the wild-type β-chain from several β-chain variants such as HbC, D, or E". Brennan *(2)* comments similarly by stating that whereas traditional methods readily detect the majority of common variants, such as HbC, HbD, or HbE, "the substitutions involved in these, and similar charge variants (Glu↔Lys, Glu↔Gln, Asp↔Asn, and Lys↔Gln) involve mass changes of 1 Da or less, and are not detectable by mass spectrometry." Kleinert et al. *(1)* also state: "Second, MS as described here is only a qualitative technique, and in particular, minor Hb fractions such as HbA_{1c} or HbA_2, which are important for diagnosis of diabetes mellitus or thalassemias, respectively, cannot be quantified."

While we agree that ESI-MS cannot detect the zero mass change mutations (Lys↔Gln and Leu→Ile), we maintain it is not necessary to resolve the variant and normal globin chains in heterozygotes to detect variants that differ in mass from normal by ±1 Da (Glu↔Lys, Glu↔Gln, Asp↔Asn, Asn→Ile). In 2003, Rai et al. *(3)* showed that variants differing by 1 Da from normal can be detected if present at >10% abundance. In that report, the normal β-chain mass was determined with a precision of 0.05 Da SD, which resulted in a 0.10 Da mass change being detectable with 95% confidence. We routinely analyze Hb on a quadrupole instrument and, owing to improved performance since 2003, generally achieve ≤0.03 Da SD on the normal β-chain when using the α-chain for internal calibration. For example, 50 normal blood samples analyzed over the last 4 months gave a mean β-chain mass of 15 867.255 Da (0.026 Da SD).