MUNI | RECETOX

P11/0290 Mýty a hoaxy – biomarkery v diagnostice

Examples of false myths

- A prolonged activated partial thromboplastin time (APTT) is always associated with bleeding

  An increase value of cardiospecific troponin always reflects an acute myocardial infarction (AMI)

  The generation of ischemia modified albumin (IMA) only occurs in the presence of myocardial ischemia

  Increased D-dimer values are diagnostic of venous thromboembolism (VTE)

  Screening of prostate cancer with prostate specific antigen (PSA) is effective to decrease cancer-related mortality and increases quality of life

  Dibucain number must be systematically assessed as part of pre-anesthesia surgical screening

  Bence Jones protein (BJP) should be assessed before administration of contrast media

  Increased concentration of lipoprotein[a] [Lp(a)] is associated with enhanced risk of mortality

  Neutrophil gelatinase-associated lipocalin (NGAL) is the ‘troponin of the kidney’

  Hyperkalemia is always a consequence of a metabolic disorder

  Laboratory values within the reference ranges are always normal and vice versa

Reference ranges

One of the mostly widespread misconception in laboratory diagnostics is that test results that fall within the ‘reference range’ are always ‘normal’, and vice versa. The notion of developing and using reference ranges (also referred as ‘reference intervals’) for interpreting any kind of measure
is indeed ancient as mankind. In laboratory medicine, this concept has pragmatically overcome the earlier notion of ‘normal values’ due to objective difficulties to establish what is normal and what is not. In brief, the reference range is conventionally described by the variation of values in a population of ‘presumably’ healthy individuals, which should be represented by at least 120 subjects.

Reference ranges II

According to sample distribution, the final calculation of the reference range can be made using a parametric statistical approach (i.e., assuming a normal distribution), or non-parametric statistical approach (i.e., assuming a non-normal distribution), or even parametric statistics after log-normal transformation of data. In the former case, e.g., a 95% prediction interval is estimated based on the mean ± 2 standard deviations (SDs). The calculated reference range should also be partitioned in separate classes when a clinical basis or logical physiological foundations exist and systematically verified once variations in analytical and/or preanalytical procedures are introduced. These rather simple and intuitive assumptions are however difficult to be appreciated by several stakeholders of laboratory services, including some hospital physicians, general practitioners, nurses and patients, wherein patient data outside the reference range are frequently perceived as axiom of 'abnormality'. It is also undeniable that the presence of 'asterisks', commonly used for flagging results falling outside the reference range, contributes to increase the chance of misinterpretation.

Reference ranges III

There are two latent and rather comprehensible limits in the statistical calculation of the reference intervals. First, the 95% prediction interval inherently excludes 5% of presumably healthy subjects, which would result as outliers, be ‘flagged’ and thereby considered as ‘abnormal’ applying the misleading conception that reference range and normal values are synonyms. However, the healthy population is only ‘presumably’ normal, since preclinical and clinical information are typically collected by short medical examination and/or questionnaires,
without more accurate testing.

APTT and bleeding

The activated partial thromboplastin time (APTT) is a global test of hemostasis, which is typically requested in combination with prothrombin time (PT) and fibrinogen for assessment of secondary hemostasis, also known as 'blood coagulation'. Pathological derangements of the delicate hemostatic balance may alternatively expose the patient to ineffective coagulation and bleeding (i.e., hypofunction), or excess clotting and thrombosis (i.e., hyperfunction). Along with mounting evidences that shortened values of APTT may reflect a prothrombotic state, prolonged values have been historically associated with a potential hemorrhagic risk. Basically, an isolated prolongation of APTT mirrors the presence of plasma inhibitors and acquired or inherited deficiencies of coagulation factors of the intrinsic pathway, thus including von Willebrand factor, coagulation factors XII, XI, IX and VIII.

APTT and bleeding II

- Although it is hence undeniable that an isolate prolongation of APTT, once preanalytical artifacts have been excluded, may reflect life-threatening abnormalities of secondary hemostasis such as von Willebrand disease, hemophilia A (i.e., factor VIII deficiency), B (i.e., factor IX deficiency) and C (i.e., factor XI deficiency), a variety of other conditions might cause abnormal prolongations. Among these, nearly half of the cases are represented by isolated factor XII deficiency (approx. 34% of cases) and the presence of lupus anticoagulant (LAC) (approx. 13% of cases). Although the former condition may be associated with remarkably prolonged clotting times, up to incoagulable APTTs, the clinical picture is not accompanied by a significant bleeding diathesis, even in patients homozygous for nearly complete factor XII deficiency (i.e., factor activity < 1%).

APTT and bleeding III

Even more interestingly, epidemiological data are in support of a putative role of factor XII deficiency in thrombosis, both venous and arterial. Whether or not this association is biologically plausible, it is unquestionable that the occasional finding of a prolonged APTT due to factor XII deficiency should not delay invasive procedures or discourage the appropriate treatment of thromboembolic accidents. The association between prolonged APTT and LAC is even more fascinating. It has been known for decades that the leading laboratory aspect of LAC lies in its ability to prolong phospholipid-dependent clotting time in vitro, thus including APTT. However, the presence of LAC is associated with a high risk of venous or arterial thrombosis, and even with pregnancy morbidity. These events globally contribute to define the antiphospholipid syndrome (APS), which treatment of choice typically remains long-term anticoagulation therapy despite the presence of prolonged APTT.

Troponiny v diagnostice kardiovaskulárních patologií

- Cardiovascular disease, including acute myocardial infarction (AMI), is the leading cause of mortality and disability in western countries. Although several biomarkers have been proposed over the past decades, cardiospecific troponin testing – with either troponin I (TnI) or troponin T (TnT) – remains the biochemical gold standard. The recent development of new immunoassays, conventionally defined ‘latest generation’ or ‘highly-sensitive’ (HS) and characterized by improved analytical sensitivity, has partially revolutionized the triage of patients with suspected acute coronary syndrome (ACS), inasmuch as measurable values of these biomarkers can now be obtained in the vast majority of presumably healthy subjects. Once analytical errors and frequent interferences such as spu rious hemolysis, heterophilic antibodies and macrotroponin have been ruled out, the optimized analytical performance of these novel immunoassays has remarkably improved the identification of minor and non-ischemic cardiac injuries.

Troponiny v diagnostice kardiovaskulárních patologií

As such, the false assumption that the cause of increased troponin values in patients with signs or symptoms of cardiac ischemia is an underlying ACS is no longer valid, wherein concentration exceeding the 99th percentile reference limit can be due to: 1) non-ischemic cardiac diseases such as cardiotoxicity from chemotherapy or poisoning, atrial fibrillation, hypertension, left ventricular hypertrophy and systolic dysfunction; 2) extra-cardiac disorders including pulmonary, renal and liver disease, systemic or localized infections and head trauma, as well as physiological conditions such as strenuous physical exercise and aging.

It is hence advisable that the clinical interpretation of HS-troponin results should be substantially revolutionized, i.e., weighted against pre-test probability, carefully troubleshooting of non-ischemic increases in condition that clinically mimic an ACS, and also taking into account the leading demographical and biological factors.

D-dimers and trombembolism

The term D-dimer is conventionally used to define a vast array of fibrin fragments generated after a thrombotic event has occurred. In brief, during activation of secondary hemostasis and subsequent fibrin generation, fibrinogen is converted into fibrin by enzymatic cleavage of the fibrinopeptides A and B. The following linkage of the C terminal tails of fibrinogen γ-chains catalyzed by activated factor XIII results in dimerization of D-domains of adjacent fibrin monomers. The proteolysis of cross-linked fibrin catalyzed by plasmin finally generates a large number of multiple cross-linked D-domains, which are heterogeneous in composition and size. The presence of D-dimer in blood thus reflects in vivo fibrin formation during blood clotting, and its measurement is conventionally used for diagnosing disseminated intravascular coagulation (DIC) and venous thromboembolism (VTE), which includes both deep venous thrombosis (DVT) and pulmonary embolism (PE). Although it is hence under - standable that any condition that may be associated with increased fibrin generation and lysis would contribute to increase the concentration of D-dimer in blood, there is a generalized misconception that increased D-dimer values always reflect the presence of blood clot(s), and that these clots are always synonyms of VTE, DIC and other typical thrombotic disorders.

D-dimers and trombembolism

Due to its peculiar biology, D-dimer concentrations are frequently increased in a large number of ‘non-conventionally’ thrombotic disorders such as Alzheimer’s disease, generalized or localized infections (e.g., sepsis and pneumonia), malignancy, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, liver disease, sickle cell disease, atrial fibrillation, recent injury and surgery, as well as in the elderly population and pregnancy . Another common misconstruction that pervades clinical practice is that D-dimer concentration should always be increased in the presence of VTE. Under some circumstances, however, non-diagnostic values of D-dimer may be present in patients with DVT and/or PE, essentially when D-dimer is tested too early or too late. Normal D-dimer values are also frequently found in patients with non-thrombotic PE.

Bence-Jones protein

The Bence-Jones protein (BJP) is a monoclonal free light chain of immunoglobin, that is typically detectable in the urine of patients with multiple myeloma, Waldenström's macroglobulinemia, monoclonal light chain-related amyloidosis, light chain deposition disease (LCDD), lymphomas and chronic lymphocytic leukemia, but that can also be idiopathic in nature (i.e., benign or of undetermined significance). The global incidence of BJP is low and globally comprised between 0.9 and 4:100000 in Western´countries. The presence in urine of monoclonal free light chain is typically due to increased production from a single clone of B lymphocytes and saturation of tubular reabsorption. The molecular mass of the protein varies widely, ranging from 5 kDa (i.e., low-molecular mass fragments), 22 kDa (i.e., monomers) and 44 kDa (i.e., dimers). The current clinical indications for BJP testing are limited to patients with serum monoclonal component at diagnosis and during follow-up, or those suspected of having a clinical or laboratory suspicion of monoclonal gammopathy.

Bence-Jones protein and contrast media

Old reports of renal impairment occurring after contrast media administration in multiple myeloma described some cases of tubular obstruction due to precipitated BJP, thus propelling a generalized belief that intravenous contrast agents may be contraindicated or place myeloma patients at unacceptable risk for developing contrast-induced nephropathy (CIN). More recent studies showed, however, that the use of the iso-osmolar agents is associated with a low incidence of CIN in the clinical setting of radiology practice, and that the incidence of CIN in patients with multiple myeloma with a normal creatinine level is low, so that the administration of contrast agent may be safe even in this patient popula-tion . It has also become clear that the precipitation of BJP should be considered highly unlikely when the newer classes of contrast media are used, and if patients are not dehydrated. As such, BJP testing before contrast media administration is no longer advisable, considering that the ionic compounds that are more likely to interact with BJP have been almost completely dismissed. A greater risk of CIN only remains in patients with impaired renal function. This has led the European Society of Urogenital Radiology (ESUR), in its guidelines on contrast media dministration, to recommend the identification of high- risk patients by assessment of glomerular filtraction rate (GFR), although no mention is made on BJP assessment.

Potassium

Hyperkalemia is a conventionally considered a life-threatening condition, in which serum potassium exceeds 5.5 mmol/L (5.0 mmol/L in plasma). Serum potassium values above 6.5 mmol/L must be handled as an emergency due to the high burden of associated mortality. Under normal conditions, only 1%–2% of the total potassium is extracellular. As this ion plays a crucial role in cellular metabolism, depolarization and tissue excitability, even modest variations of extracellular levels can produce remarkable clinical effects, thus triggering cardiac dysrhythmia and arrest. Most physicians, and even some laboratory professionals, are persuaded that almost exclusive sources of hyperkalemia include renal impairment, medication side effects, hypoaldosteronism and massive tissue breakdown such as in patients with rhabdomyolysis, thus overlooking the fact that an increased potassium concentration in serum or plasma may also be due to preanalytical artifacts.

Potassium

Although the prompt recognition of the high risk associated with this electrolyte abnormality should stimulate preventive and therapeutic measures, unnecessary treatment of spurious hyperkalemia (also known as ‘pseudohyperkalemia’) may be associated with significant morbidity and mortality. The leading cause of pseudohyperkalemia is represented by spurious hemolysis, i.e., erythrocyte injury or breakdown during collection, transportation and preparation of blood samples, most frequently in patients with leukocytosis and/or thrombocytosis. An additional and often unrecognized cause of pseudohyperkalemia is the transferal of blood collected in primary blood tubes containing K 2 or K 3 ethylenediaminetetraacetic acid (EDTA), into those with no addictives or containing lithium-heparin and further referred for clinical chemistry testing. It is thereby essential that physicians, nurses and laboratory professionals clearly recognize that hyperkalemia is not always due to metabolic imbalance or disease(s), but may often be caused by preanalytical artifacts, with little clinical significance for patient’s health.

PSA and prostate cancer

Prostate cancer is the second most common malignancy in males, with an estimated frequency of 29% of all cancers, as well as the second cause of death for cancer in the male gender (i.e., 9.3% of all deaths). There is mounting debate about the clinical effectiveness of prostate cancer screening by means of prostate specific antigen (PSA) testing. More specifically, the American Cancer Society

(ACS) recommends that men make an informed decision with their physician about the opportunity to be tested, since the current evidence is still uncertain as to whether the potential benefits of screening would ultimately outweigh the harms of testing and treatment. The US Preventive Services Task Force has recently concluded that PSA screening has resulted in small to no reduction of mortality for prostate cancer, but is associated with harms related to subsequent evaluation and treatments,

some of which are deemed unnecessary. As regards the European Association of Urology guidelines, it has recently been concluded that there is insufficient evidence to warrant widespread population-based screening by PSA so far.

PSA and prostate cancer

As regards the last two measures, two recent metaanalyses have almost simultaneously concluded that assessment of p2PSA or phi not only improves the accuracy of prostate cancer detection in comparison with PSA, but also carries additional clinical advantages, as reflected by the greater ability to identify more aggressive cancers and thus patients harboring more clinically relevant prostate cancers and increased likelihood of death. Along with the widely-used total and free-PSA, genetic analysis entailing four polymorphisms of KLK3 gene encoding for kallikrein-related peptidase 3 (rs2569733, rs2739448, rs925013 and rs2735839) and one polymorphism of the SRD5A2 gene encoding human steroid 5 α-reductase type 2 (rs523349), was shown to increase the diagnostic accuracy of free-to-total PSA ratio (fPSA/tPSA), especially in patients with fPSA/tPSA values comprised between 11% and 14.5%. Whether or not these findings will translate into official guidelines and recommendations, it is, however, essential to clearly emphasize that several doubts remain about the efficacy of PSA-based screening.

PSA and prostate cancer

Taken together, these facts legitimate the hypothesis that although PSA screening may be indeed effective to detect prostate cancer, its clinical efficacy for decreasing prostate cancer mortality is largely questionable and potential offset by economical and clinical harm.

This is mainly attributable to the fact that a large number of prostate cancer, up to 50%, may be classified as indolent and slow-growing, so that their surgical ablation would not improve the outcomes, but rather expose the patients to a variety of side effects such as incontinence, impotence, bleeding and septic complications. To overcome the latent drawbacks of PSA-based screening, some alternative strategies have been proposed, including calculation of PSA velocity and density, ratio between free and total PSA, the use of age-specific reference ranges, as well as assessment of isoform p2PSA or the calculation of the Prostate Health Index (phi: [p2PSA/free PSA] × √total PSA).

Conclusion

The contribution of laboratory testing in science and medicine is almost unquestionable. The number of tests is continuously expanding, so that it becomes challenging to stay abreast of all recent developments and eventually dismiss redundant or obsolete analyses. These aspects have contributed to proliferation of false myths and legends, consuming valuable human and economic resources, and jeopardizing the clinical reasoning. It is obvious that the paradigmatic cases described in this article are not intended to be comprehensive, since other peculiar examples have been overlooked. However, since medical science and laboratory medicine have to frequently fight against false beliefs, we believe that these cases may help remove some mysticisms in laboratory diagnostics.

Děkuji za pozornost