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Editorial

Cancer diagnosis: from dogs to DNA or from DNA to dogs?

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Laboratory medicine plays an increasingly important role in the management of cancer patients (1). The involvement of laboratory medicine in clinical oncology is based on the concept of biomarkers. A biomarker may be defined as a parameter that can be detected in the laboratory and is associated with malignant transformation or tumor progression. The utilization of biomarkers encompasses predicting the subject's risk of cancer [e.g., determining BRCA1 mutation to identify individuals with high risk of breast and ovarian cancer (2)], contributing to the diagnosis [e.g., utilization of tumor markers for pre-operative diagnosis of ovarian cancer (3, 4)], predicting the prognosis and response to therapy, or monitoring the therapeutic response or detecting the recurrence. Biomarkers are also used to assess the toxicity of anti-cancer therapy (5).

In the current issue of *Clinical Chemistry and Laboratory Medicine* Lippi and Cervellin (6) present a rather provocative topic that may seem in stark contrast with the prevalent "conventional" use of laboratory diagnostics in cancer medicine. The authors summarize the experience with canine olfactory detection of cancer from early anecdotal reports to subsequent prospective studies. The scientific foundations of canine olfactory detection are also thoroughly reviewed and the pitfalls of this approach in cancer screening, including the problems already identified, or potential confounding issues yet to be investigated are also addressed.

The interest in cancer screening was prompted mainly by dramatic differences in survival rates of patients with malignant tumors according to the stage at diagnosis. Early detection through screening programs has resulted in increased survival of patients with breast cancer, cervical cancer or prostate cancer (7, 8). In addition, the therapy of advanced tumors is much more expensive compared to tumors detected at an early stage. Thus, early detection markedly increases cost-effectiveness of therapy. Unfortunately, for the malignant tumors of most primary locations an effective screening still remains an unfulfilled dream.

The idea of using dogs for cancer screening may seem odd at first glance and evokes to some readers the specter of alternative medicine. Physicians who have some experience with uses (or rather abuses) of so-called alternative medicine may be alarmed by the perspective of barking of a dog in a doctor's office or clinical laboratory forming the basis of important medical decisions. However, everyone is familiar from daily life with the use of canine olfactory detection to detect explosives or illicit drugs, and the use of canine olfactory detection for security or forensic purposes is a generally accepted practice. The use of dogs is well established in forensic medicine, for example, for searching for human remains (cadaver dogs) (9) or in fire investigations (10). Why then should this "technology" not be acceptable for diagnosis of today's most dreaded disease? Frankly speaking, for most tumors we do not have an effective alternative to offer, and it is not ethical to reject a method only because it does not fit our idea of a screening method. A number of laboratory assays have been established relying on sample interaction with complex organisms (e.g., Limulus amebocyte lysate test that utilized horseshoe crab lysate to detect the presence of endotoxin), so why should we exclude the species *Canis familiaris*?

The potential of canine olfactory detection in cancer diagnosis could be illustrated by a recent Japanese study in patients with colorectal cancer (11). Colorectal cancer is one of the leading causes of cancer-related death. When detected early, the cure rate is high, but the curability decreases dramatically with advancing stage. The positive predictive value of fecal occult blood test, the screening method currently in use, is low. Additional studies are needed to clarify positive findings from the screening test, colonoscopy or barium enema, imposing a certain burden both on the patients and the system. The results of the study by Sonoda et al. (11) show that the sensitivity of canine olfactory detection when compared with colonoscopy findings was over 90%, while the specificity for stool and breath samples was 99%. These results may look almost too good to be true. In any case, more investigations of canine olfactory detection for cancer screening are needed, and the method still has a long way to go before it can be used in clinical medicine. The sensitivity, specificity, reproducibility and the fact of whether the method actually saves lives have to be established.

The concept of utilization of canine olfactory detection in the diagnosis of cancer may seem to be in contrast with the utilization of molecular diagnostics in cancer medicine. We can, for example, contrast the study by Sonoda et al. (11) with a study examining the potential of early detection of the same tumor, colorectal cancer, published recently by the same authors who present in the current issue the review on canine olfactory detection of cancer (12). The authors investigated the potential of measuring cell-free DNA in the sera of patients with colorectal cancer, polyps and normal subjects. Cell-free DNA concentrations were markedly increased in patients with colorectal cancer, and, more importantly, increased cell-free DNA concentrations separated cancer patients from normal subjects much better than the levels of an established

biomarker, the carcinoembryonic antigen. Nevertheless, the utilization of the measurement of serum cell-free DNA using the suggested cut-off for colorectal cancer screening would still miss many cases of cancer.

Does the fact that respected laboratory scientists with extensive publication records on molecular detection of cancer write a review on cancer detection by dogs mean that the molecular approach to cancer screening has exhausted its potential and kennels would replace laboratory benches? Certainly not, and both approaches could very effectively complement each other.

Cancer screening (or secondary prevention) is typically a two-phase process. A screening test as the first step usually selects individuals in whom the diagnosis of cancer has to be confirmed by more extensive investigations in the second step. Using trained dogs to identify patients who may harbor occult tumors would identify patients for more extensive (and also more expensive) testing that would include analyses on the molecular level (e.g., looking for tumor-specific DNA changes). On the other hand, screening the whole population would be demanding in terms of logistics, and some pre-selection process would be needed, for example, selecting patients based on hereditary predisposition that is identified by DNA testing (e.g., in the case of colorectal carcinoma, presence of mutations in hMSH2 or hMLH1 genes). Thus, the diagnostic process would have to move in both directions, from dogs to DNA and from DNA to dogs.

Obviously, at this moment the concept of incorporating canine olfactory detection into cancer screening programs is still a fantasy that may or may not become reality in the future. More work needs to be done on the reproducibility of this testing that is, for certain, more "operator-dependent" than conventional laboratory methods. Even if validity of this approach is proven by a large scale experience, logistical problems could pose a major obstacle. The number of people threatened by cancer is orders of magnitude higher than the number of victims of violent crime or terrorism, and training a sufficient number of dogs could be a serious problem that could be compounded, in some countries, by cultural issues.

In the past, it has not been easy for new ideas for cancer screening to enter clinical practice, as exemplified the story of the Pap smear (8). The idea of using vaginal smears for early diagnosis of cervical cancer was first presented by Papanicolaou in 1928, but only became generally accepted more than a decade later (8). Only time will tell whether canine olfactory detection follows the path of the Pap smear, remains in obscurity or even falls into oblivion.

As outlined above, even if canine olfactory detection could become a reality one day, it will have to be complemented by more conventional methods, including the methods of molecular diagnostics. This more conventional approach of utilization of laboratory medicine in the management of cancer patients is highlighted in the current issue of *Clinical Chemistry and Laboratory Medicine* by several papers (2, 13, 14) and in an article by Soh et al. recently published in this *Journal* (15).

The paper by Buszewski et al. (14) presents data that may be regarded as bridging the gap between "classical" diagnostic methods and canine olfactory detection. The authors analyzed volatile organic compounds released by non-small-cell lung cancer (NSCLC) explants cultured in vitro and in the breath of NSCLC patients. Marked differences in volatile organic compound concentrations were noted in the breath of patients and controls (14). It is possible that these differences are detected by animals with a very sensitive olfactory system, for example, dogs. Currently, most biomarkers are determined in the serum (or plasma) and tumor tissues, and less frequently in other body fluids (16) or urine (17, 18). In agreement with earlier publications (19), the paper by Buszewski et al. indicates that exhaled air may represent another source of samples for biomarker determination. In particular, the utilization of exhaled air is attractive from a point of view of repeated sampling.

Breast cancer is the most common malignancy in women. Considerable progress that has been achieved during the last two decades in the genetics of breast cancer is reviewed by Poumpouridou and Kroupis (2). The identification of subjects with a high risk of malignancy is of obvious importance for selection of a population for cancer screening. Moreover, the presence of BRCA1 mutation may be a predictive biomarker of response to certain agents, including platinum compounds or poly-(ADP-ribose)-polymerase 1 (PARP1) inhibitors that represent a targeted treatment in patients with these tumors. BRCA1 and BRCA2 mutations are characteristic of different populations, and it has been possible to trace the movement of founder mutations many centuries back. For example, it is interesting to note that single founder mutation (c.5266dupC) is the most common BRCA1 mutation across the Eastern or Central part of Europe in diverse countries, including Poland, Russia, the Czech Republic, Hungary or even Greece (20). Poumpouridou and Kroupis also review the data on other genes that when mutated increase the susceptibility to breast cancer, including recently discovered PALB2.

Improved early detection is not the only cause of decreasing cancer mortality for many tumors. Cancer mortality rates are also decreasing because of better therapy for systemic disease. While in the second half of the 20th century systemic therapy has relied on the use of cytotoxic drugs that indiscriminately kill rapidly proliferating cells, more targeted treatments have been at the forefront of research in the past two decades. The earliest targeted treatments were hormonal therapy for breast cancer. The clinical use of hormonal therapy has highlighted the requirement to identify the presence of the target, in this case the estrogen or progesterone receptor, in tumors of individual patients. A number of studies summarized in meta-analyses revealed that the administration of hormonal therapy is effective only in patients with tumors characterized by high expression of estrogen receptor (21).

After the approval in 1997 by the US Food and Drug Agency of rituximab, monoclonal antibody against the CD20 antigen expressed on the surface of lymphoma cells, molecularly targeted agents were developed for the therapy of common solid tumors, for example, NSCLC. Targeted therapies, including small-molecular-weight epidermal growth factor receptor (EGFR) inhibitors erlotinib and gefitinib (22, 23) and anaplastic lymphoma kinase (ALK) inhibitor crizotinib (24), have demonstrated activity that was, however, restricted to sub-groups of patients with advanced NSCLC selected by

the presence of specific mutations. The presence of mutations in EGFR or ALK genes represents biomarkers that are crucial for patient selection for the targeted treatment. In patients with advanced NSCLC, the diagnosis is often made based on cytology and the patient is subsequently treated with systemic therapy. Unfortunately, a sufficient sample for the identification of specific tumor DNA mutation may not be available in most of these patients. Importantly, it has recently been reported that there seems no heterogeneity in the distribution of EGFR mutations among the primary tumor and the metastases (25). The data presented by Cho et al. (13) indicates that body fluids, for example, pleural effusion, ascites or cerebrospinal fluid, could represent an alternative sample source for the determination of tumor mutation status and that this approach may be feasible in clinical practice. In this pilot study, EGFR mutations were detected in 18 out of 32 specimens. The therapy was started based on finding of mutations in 12 patients and in two patients treatment was discontinued because a resistant mutation was discovered. These results have potentially a great impact on clinical practice and may serve as an example of utilization of pharmacogenetics in the practical management of cancer patients.

Different aspects of pharmacogenetics in cancer treatment are comprehensively reviewed by Soh et al. (15). As the authors state, genetic biomarkers are used in cancer medicine with two general aims; to identify the patients at risk for treatment toxicity, and to guide the selection of therapy based on tumor sensitivity determined by predictive biomarkers or risk of recurrence associated with prognostic biomarkers. Genetic biomarkers that predict toxicity are represented by germline mutations of genes involved in the metabolism of anti-cancer agents. Predictive biomarkers could be exemplified by the papers of Cho et al. (13) or Poumpouridou and Kroupis (2) that are discussed above. EGFR mutations are determined to predict the efficacy of erlotinib or gefitinib in NSCLC, while the presence of BRCA1 mutations might select the patients who could benefit PARP inhibitors. Yet these two cases are different, since EGFR mutations are acquired and are determined in tumor cells, while BRCA1 mutations are germline and could be determined in any nucleated cell. Predictive biomarkers could also be polymorphisms in genes governing the metabolism of certain drugs, for example, tamoxifen. Biomarkers are also used to identify the patients with high risk of recurrence. Adjuvant treatment results in a proportional reduction of risk. Based on the risk of recurrence, the absolute benefit of a given therapy differs greatly. Empirical models are now used to calculate the individual risk of recurrence in order to guide the decision of whether or not to administer adjuvant treatment. Gene expression profiles have gained a wide use in the management of early breast cancer (e.g., Oncotype Dx or MammaPrint).

The papers in the present issue of *Clinical Chemistry and Laboratory Medicine* clearly illustrate challenges that both laboratory medicine and clinical specialties are facing. Despite substantial progress in recent years we still have for most cancers no tests that would allow population-wide screening. Although the indication for targeted treatment critically relies on the identification of appropriate molecular targets or changes in the targeted pathways, in clinical practice we also often lack that information, and targeted therapies are administered to an unselected population. The stakes are high as untreated cancer is a fatal disease, and all leads need to be followed in the investigation of better diagnostic methods for the benefit of cancer patients. To save patients lives we should explore every possible option, even if this means going from using dogs for screening to DNA analyses to confirm the presence of tumor or DNA analysis to identify patients with hereditary predisposition to cancer who may then be screened by dog.

References

1. Melichar B, Plebani M. Laboratory medicine: an essential partner in the care of cancer patients. Clin Chem Lab Med 2011;49:1575–8.
2. Poumpouridou N, Kroupis C. Hereditary breast cancer: beyond BRCA genetic analysis; PALB2 emerges. Clin Chem Lab Med 2012;50:423–34.
3. Montagnana M, Danese E, Ruzzenente O, Bresciani V, Nuzzo T, Gelati G, et al. The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in women presenting with pelvic mass: is it really useful? Clin Chem Lab Med 2011;49:521–5.
4. Kim YM, Whang DH, Park J, Kim SH, Lee SW, Park HA, et al. Evaluation of the accuracy of serum human epididymis protein 4 in combination with CA125 for detecting ovarian cancer: a prospective case-control study in a Korean population. Clin Chem Lab Med 2011;49:527–34.
5. Cardinale D, Salvatici M, Sandri MT. Role of biomarkers in cardioncology. Clin Chem Lab Med 2011;49:1937–48.
6. Lippi G, Cervellin G. Canine olfactory detection of cancer versus laboratory testing: myth or opportunity? Clin Chem Lab Med 2012;50:435–9.
7. Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360:1320–8.
8. Michalas SP. The Pap test: George N. Papanicolaou (1883–1962): a screening test for the prevention of cancer of uterine cervix. Eur J Obstet Gynecol Reprod Biol 2000;90:135–8.
9. Oesterhelweg L, Krober S, Rottmann K, Willhoft J, Braun C, Thies N, et al. Cadaver dogs – a study on detection of contaminated carpet squares. Forensic Sci Int 2008;174:35–9.
10. Nowlan M, Stuart AW, Basara GJ, Sandercock PM. Use of a solid absorbent and an accelerant detection canine for the detection of ignitable liquids burned in a structure fire. J Forensic Sci 2007;52:643–8.
11. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, et al. Colorectal cancer screening with odour material by canine scent detection. Gut 2011;60:814–9.
12. Danese E, Montagnana M, Minicozzi AM, De Matteis G, Scudo G, Salvagno GL, et al. Real-time polymerase chain reaction quantification of free DNA in serum of patients with polyps and colorectal cancers. Clin Chem Lab Med 2010;48: 1665–8.
13. Cho MC, Choi CM, Jang S, Jang S, Park CJ, Chi HS, et al. Direct sequencing in cytological specimens as a useful strategy for detecting EGFR mutations in non-small cell lung cancer patients. Clin Chem Lab Med 2012;50:565–72.
14. Buszewski B, Ulanowska A, Kowalkowski T, Cieslinski K. Investigation of lung cancer biomarkers by hyphenated separation techniques and chemometrics. Clin Chem Lab Med 2012; 50:573–81.

15. Soh TI, Yong WP, Innocenti F. Recent progress and clinical importance on pharmacogenetics in cancer therapy. Clin Chem Lab Med 2011;49:1621–32.
16. Yamada S, Tabata C, Tabata R, Fukuoka K, Nakano T. Clinical significance of pleural effusion mesothelin in malignant pleural mesothelioma. Clin Chem Lab Med 2011;49:1721–6.
17. Melichar B, Karlicek R, Tichy M. Increased urinary nitrate excretion in inflammatory bowel disease. Eur J Clin Chem Clin Biochem 1994;32:3–4.
18. Melichar B, Urbanek L, Krcmova L, Kalabova H, Svobodova I, Dragounova E, et al. Urinary neopterin in patients with ovarian cancer. Pteridines 2006;17:145–53.
19. Dodig S, Richter D, Zrinski-Topic R. Inflammatory markers in childhood asthma. Clin Chem Lab Med 2011;49:587–99.
20. Hamel N, Feng BJ, Foretova L, Stoppa-Lyonnet D, Narod SA, Imyanitov E, et al. On the origin and diffusion of BRCA1 c. 5266dupC (5382insC) in European populations. Eur J Hum Genet 2011;19:300–6.
21. Early Breast Cancer Trialists Collaborative Group. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011;378:771–84.
22. Shepherd FA, Pereira JR, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. Erlotinib in previously treated non-small-cell-lung cancer. N Engl J Med 2005;353:123–32.
23. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008;372:1809–18.
24. Kwak EL, Bang YJ, Camidge R, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–703.
25. Yatabe Y, Matsuo K, Mitsudomi T. Heterogenous distribution of EGFR mutations is extremely rare in lung adenocarcinoma. J Clin Oncol 2011;29:2972–7.

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