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Significance of Diagnostic Needle Biopsy for the Development of Inflammation, Tumour Progression and Metastasis

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Abstract

A recurring and frequently neglected problem is that the current cancer diagnostic procedure sometimes is time-consuming, may be unnecessarily costly and inconvenient for the patient.

In the present review we discuss the current status and recent important studies that highlight potential risks for adverse effects that may be associated to the prevailing diagnostic core needle biopsy (CNB) procedure. One problem discussed in the literature is seeding of tumour cells as a side effect of CNB. It has been shown to occur in mouse models but studies in human tumours have emerged contradicting results.

We suggest that the procedures for tumour sampling by CNB have to be improved primarily to reduce the inflammation in biopsy. Minimally, triggering tumour cells as observed in connection to biopsy. Minimally traumatic fine needle aspiration biopsy (FNAB) technique associated with implementation of new sensitive methods for biomarker analysis is suggested as an alternative. In the near future, clinical validation of biomarkers for accurate diagnosis and therapy selection will be pivotal for the implementation of personalized and precision cancer medicine.

Keywords: Tumours; Fine needle aspiration biopsy; Breast cancer; Core needle biopsy; Surgical biopsy; Cytokine IL-6; Inflammation

Introduction

In the present review we use breast cancer as a model in discussing biopsy techniques, wounding, inflammation and tumour cell seeding. Breast cancer is the most prevalent female cancer worldwide and despite decades of intense research and development of various therapies, the long term survival in breast cancer has not improved radically [1].

Therefore, there is a valid reason to once again carefully examine all components of the chain from early detection and diagnosis to treatment and relapse after a long follow up period to examine in detail what can be improved. Diagnostic sampling is an important step of this chain, whose role we do not pay enough attention and which is the main focus of the review presented. It is of course of outmost importance to obtain an accurate and objective analysis of any suspicious lesion in order to generate the best support for the selection of treatment. This is not possible today without a cell or tissue biopsy.

The main question we aim to scrutinize is how the biopsy currently is obtained, if there are any potential risks associated with the procedure, and if so - what can be done to improve current practices and to minimize the risk for complications and at worst distant seeding of cancer cells.

Methods for Cell or Tissue Biopsy

Surgical biopsy

Open surgical biopsy can be subdivided into incisional and excisional biopsy. Incisional biopsy means removal of a part of a suspicious lesion for histological diagnosis, whereas excisional surgical biopsy means intended removal of the entire lesion. Because of the globally rapidly expanding introduction of diagnostic CNB techniques surgical biopsy, especially incisional biopsy is used at a decreased rate. The extension of tissue trauma caused by incisional surgical biopsy is mainly related to the size of the removed tissue and is significantly larger than that of needle biopsy. Complications due to incisional biopsy, that has been the prevailing method for example in China, has resulted in an increased use of CNB to avoid unnecessarily high frequency of surgery associated problems [2].

Fine needle aspiration biopsy (FNAB)

Fine needles range from 0.4 to 0.7 mm (26-21 gauge) outer diameter and lengths vary from 1 cm (sampling of *e.g.* skin lesions) to 20 cm (sampling of *e.g.* prostate lesions). During several decades, the one-handed syringe pistol handle for 10 to 20 ml disposable plastic syringes designed in the 1960s by Sixten Franzén is generally in use [3]. The course of action regarding aspiration of cellular material from solid lesions varies significantly dependent on configuration and firmness of the lesion and the experience and skill of the investigator [4]. When the needle is inserted into the lesion, the investigator guides the needle in average five to ten times back and forth through the lesion. The total number of sampling procedures varies but ranges in average between

two and five. Palpable lesions are generally biopsied directly whereas non-palpable lesions are biopsied guided with help of various modalities of imaging techniques *e.g.* ultrasound, stereotactic devices and MRI [3]. The advantages of FNAB is that the sampling procedure causes a minimum of tissue trauma (explained by the diminutive amount of absorbed energy), is extremely patient friendly and that conclusive diagnostic information may be obtained within less than half an hour performing quick staining of the aspirated cells on cytological smears. The main disadvantages are that the method does not produce a similar level of diagnostic results as compared to CNB [5] and that diagnostic accuracy is highly related to the proficiency of an experienced cytopathologist. Since many years, the lack of experienced cytopathologists hampers the utilization of FNAB. On the other hand, development of ultrasensitive molecular analysis may increase future FNAB based diagnostics. This opportunity will be discussed below.

Core needle biopsy (CNB)

In core needle biopsy technique, larger hollow needles are used compared to FNAB. The outer diameter of CNB needles ranges generally from 1.3 to more than 3 mm. The sampling instrument is an automated cutting biopsy gun with a spring-loaded sampling device [6]. The total number of sampling procedures varies but ranges in average between three and ten, or more, depending on the size of the lesion. Local anaesthesia is generally used and a little cut in the skin has to be made when sampling from *e.g.* breast lesions is performed. Vacuum-assisted biopsy (VAB) is frequently used in connection to CNB technique in breast diagnostics and reduces the ballistic spring-loaded devise effect allowing multiple samplings with only one insertion of the needle [7]. After insertion by VAB the needle will stay almost at the same place but is rotated during the biopsy procedure and the created low pressure will suck tissue material into the needle. This biopsy technique was introduced in the early 1990s and the main advantage of CNB based diagnostics is the ability to sample a sufficient amount of tissue for histological analysis which improves diagnostic accuracy including the differentiation between *in situ* and invasive cancer. Correct diagnosis compared to pathology analysis after surgery was significantly higher for 8 gauge VA-CNB as compared to 11 gauge regarding benign lesions and VAB reduces the side effect of higher energy used by 8 gauge CNB [8]. Generally, the amount of energy density, the time peak value including the final pressure re-balancing and even cavitation due to ballistic effects can be important factors regarding development of tissue trauma and tumour cell dissemination. [9]

Potential physical problems associated with current performance of CNB

The potential problems associated with needle biopsy have been debated for a long period of time. One meta-study concludes that no general increase of distant metastasis can be found after CNB [10]. In breast cancer, despite frequently observed cell displacement, no increased morbidity has been observed after CNB [10]. However, very few studies have been designed to exclude the possibility of CNB induced metastasis and strategies have been suggested to prevent tumour cell seeding [11].

Among the drawbacks of CNB are frequently observed widespread tissue trauma and the difficulty of precision placement of the needle which is a well-recognized disadvantage of core needles equipped with spring-loaded devices. Usually, the core needle is inserted using a spring loaded-device at an accelerating speed of 4-10 meters per second. With high needle velocity several complex phenomena might occur, such as standing wave formation, micro-streaming, energy conversion, pumping effects and time for balancing including transports. In analogy with ballistics of a bullet from a gun, the temporary cavity-channel produced will be considerably larger than the diameter of the needle and the kinetic energy induced by the shot must to a great amount be absorbed by the tissues [12]. In addition, an increased number of shots, sometimes up to about twenty, correlate with an increased frequency of clinical complications [13].

Therefore, it is likely to assume that the total extent of tissue trauma is related to the deposed energy, which in turn is proportional to the number of shots and the square of both needle velocity and needle diameter.

Finally, damaged blood vessels will cause a semi-static lesion pressure which might include a significant volume having a pressure (in the order of 120 mmHg) which in turn can cause emission of blood and cellular material during the low blood pressure phases, until the pressure is again balanced. These factors are likely to play a crucial role behind the observation that tumour cell displacement is detected in more than one third of the patients after CNB [14]. In contrast to CNB, the diameter of the FNA needle is ~2-5 fold smaller and the average needle velocity is >100 fold slower, and thus offers an attractive, minimal trauma technology for sampling of tumour material.

Potential risks and consequences followed by CNB

Numerous reports confirm needle tract seeding after biopsy, a complication which may be avoided to some extent by applying appropriate precautions [15]. A reasonable consequence of needle tract seeding would be a transient increase of circulating tumour cells (CTC) after CNB. However, few reports have studied this in detail by paired testing, *i.e.* examination of each patient before and after biopsy. One study reported a significant increase of CTC markers detected by reverse-transcriptase polymerase chain reaction (RT-PCR) after colon biopsy [16]. Haematogenous dissemination of epithelial cellular markers has also been observed only minutes after prostate biopsy [17].

An increase of CTC should be taken serious and several reports show that the concentration of CTC may be used as predictors of metastatic progression and treatment efficacy [18]. When CTC survive circulation, they may finally reach the bone marrow as disseminated tumour cells, where they may enter a dormant state and escape chemotherapy [19]. It is known that premalignant tumour cells can disseminate and remain dormant for long periods of time and after long time give rise to metastases [20]. It has been reported that a majority (~62%) of breast cancer patients get recurrences several years after the 5-year survival mark, and do not survive [21]. The biology of minimal residual cancer cells and cancer cell dormancy is an emerging area of research and there are many questions waiting for answers. Meanwhile, we may review current biopsy routines to find strategies for the study of biopsy associated complications, with the aim to minimize the number of dormant cancer cells displaced at distant sites.

Mouse models for studies of biopsy associated complications

Mouse models may provide important clues on the possible mechanisms underlying tumour cell dissemination and potential metastasis associated with biopsy. Using a nude mice model, various tumour tissue manipulations were tested, including compression,

biopsy, laser treatment and complete resection. It was shown that biopsy caused a ~60-fold increased release of CTC [22]. In contrast, compression causes only a minor release of CTC and therefore the magnitude of CTC released will be one important parameter to record when biopsy methods are compared.

Similar results were obtained using a breast cancer BALB/c mouse model. Mathenge *et al.* recently showed that CNB not only cause a >3-fold increase of CTC but also enhances the frequency of lung metastasis [23]. In addition, immune cell populations and molecular markers were monitored at various time points after biopsy. After 3 days, a significant decrease of macrophages, CD4+ and CD8+ T-cells were observed, paralleled by an increase of myeloid-derived suppressor cells in the tumour tissue. Already after 3 hours post biopsy, TGF+/SOX4 associated epithelial-mesenchymal transition (EMT) markers and pro-inflammatory factors like e.g. CXCL2, CCL3, S100A8 and COX2 were increased followed by an increase of TNFα (after 24 hours). Taken together, data presented in this study show that CNB causes an acute inflammatory response followed by tumour infiltration, immune suppression, EMT, CTC release and finally lung metastasis.

An acute inflammatory response induced by 2 mm punch biopsy needles has also been shown by Hobson *et al.* using the MMTV-PyMT mouse model [24]. In this study, the increase of lung metastasis was followed by an increase of the pro-inflammatory cytokine IL-6. Interestingly, IL-6 knock-out animals did not show and increased number of lung metastasis after biopsy and animals receiving anti-inflammatory Ibuprofen® treatment pre- and post-biopsy, showed a decreased number of lung metastasis.

Microenvironment, inflammation, tumour progression and metastasis

The important role of tumour microenvironment and inflammation are emerging areas of research [25] and the tumour promoting role of inflammation in cancer is today well established. Moreover, the inflammatory microenvironment and hypoxia has also been shown to be a driving force in the increase of mutations and genetic instability [26]. Inflammatory breast cancer is very aggressive and the prognosis is poor despite advanced therapy [27]. Interestingly, high levels of CTC which is frequently observed in inflammatory breast cancer can be reduced radically after treatment by statins, which has an anti-inflammatory effect. An increasing number of studies have confirmed that aspirin and non-steroidal anti-inflammatory drugs are associated with improved breast cancer survival [28]. Some of the results from the MMTV-PyMT mouse model discussed above are thereby supported by clinical observations.

Clinical relevance of possible complications after CNB

Taken together, tissue trauma including cell damage, cell translocation, blood vessel destruction and acute inflammation will inevitably have detrimental consequences for the tumour microenvironment. With the background of recent research on mouse models after CNB and increased understanding of the role of tumour microenvironment these aspects has to be studied in clinical investigations. Why is there so far no strong evidence regarding complications of CNB in breast cancer?

In prostate cancer diagnostics, several complications have been recorded as a consequence of transrectal CNB. However, the focus has primarily been oriented towards complications associated to bacterial infections [29]. In breast cancer, inflammatory reactions have been observed to support tumour growth and supply tumour stem cells to survive treatment by natural selection [30,31]. An important factor in the immunological response to tissue wound is the macrophage polarization from M1 to M2, which may accelerate tumour progression as observed in oral squamous cell carcinoma [32]. Weber *et al.* conclude in this report that M2 polarisation is a consequence of preoperative tumour biopsies.

Preclinical studies in mouse models support the concept that acute inflammation caused by sampling interventions plays an important role regarding the risk of peripheral metastases. This has been studied in a review including mouse models and hence, biopsy 'wounding' induced risk of metastases. However, this devastating side effect may be reduced by prophylactic administration of anti-inflammatory medication (*e.g.* ibuprofen) at the time of the biopsy [33].

Furthermore, SOX4 overexpression, which induces epithelial-mesenchymal transition, was correlated with triple-negative breast cancer subtypes [34]. Breast cancer represents a very heterogeneous group of cancers and the grade of malignancy shows a very wide range [35]. At the moment prior to diagnostic biopsy we cannot know if a lesion is benign, relatively low malignant or highly malignant. The size of the tumour, metastatic status and age of the patient are some other factors which have impact on the prognosis. The acute inflammatory condition followed by CNB represents an additional and important perspective and will require careful clinical evaluation regarding risk for cancer cell progression and metastasis.

The way forward: Time to upgrade how we view and do diagnostic biopsy

In spite of the fact that numerous studies have shown that diagnostic biopsy cell- or tissue sampling from solid malignancies is associated with increased risk of tumour cell dissemination and also local and/or distant tumour growth, traumatic biopsy methods are still globally used in order to receive a final diagnosis. The main reason is the need of tissue based morphologic, immune histochemical and molecular marker based diagnostic analyses in order to be able to make tumour specific treatment decisions. Another decisive reason is the difficulty to differentiate between spontaneous and biopsy related tumour cell dissemination, the influence of time, type of treatment and grade of malignancy of the tumour. However, in spite of remaining uncertainties it is generally accepted that biopsy related tumour cell dissemination may be observed in highly aggressive cancer types, *e.g.* malignant melanoma, ovarian cancer and pancreatic cancer. Here it is recommended that biopsy should be avoided. The same may well be true regarding *e.g.* the subgroup of highly malignant breast or prostate cancers. This fraction of cancer subtypes may vary from approximately 10 - 45% which means that the remaining fraction of patients with low malignant tumours may develop metastases after 10 - 15 years, making it difficult to reveal correlation to biopsy caused cancer cell dissemination.

At present there is no available reliable objective method to determine tumour aggressiveness and thus to estimate the risk of biopsy induced tumour cell spread except performing analysis of tumour specific cell- or tissue samples. In order to minimize the risk of tumour cell spread caused by biopsy tumour tissue sampling and at the same time guarantee the analysis of tumour growth and grade of tumour malignancy, there is an opportunity to make use of newly developed cell- and tissue sampling methods *i.e.* imaging guided

precision placement of the biopsy needle reducing the number of biopsies combined with anti-seeding equipped biopsy needles significantly diminishing the risk of tumour cell spread [36]. In addition, in view of recent reports discussed above, it seems very reasonable to offer patients anti-inflammatory drugs pre- and post-biopsy.

The present review supports that FNAB is associated with a lower risk of biopsy associated metastasis, so why do we not use FNAB more frequently today? As mentioned, there is a lack of experienced cytopathologists. At the same time, new ultrasensitive molecular methods have been developed and the scarce material obtained by FNAB is not any longer a limiting factor for advanced analysis. Already a decade ago it was possible to analyse gene expression profiles from FNAB samples [37], but this is not yet routine. Analysis of protein biomarkers would be preferable and also comparable to current low-plex immuno-histochemical based profiling. Recently Ullal *et al.* showed that FNAB samples can be used for very sensitive 88-plex protein analysis, not only for diagnostic purpose but also to guide therapy [38]. This example of recent development of advanced analysis is very promising and opens new opportunities for longitudinal follow up of therapy response and if needed, rapid adjustments of ongoing therapy. We have recently initiated tests using analysis of FNAB samples by proximity extension assay, an ultrasensitive and highly specific multiplex protein analysis method which also may be more cost effective compared to other profiling methods [39].

Conclusion

We conclude that we today have new unique opportunities to perform molecular diagnostics in FNAB samples. With these methods, we will have not only an at least equivalent diagnostic information compared to current practices, but also a more objective and extended molecular information to guide therapy selection and support the development of new therapies. At the same time, we get the opportunity to validate new potentially valuable biomarkers in clinical materials with minimal consumption of precious patient material. There are several biomarker candidates that may be used to determine *e.g.* tumour aggressiveness and sensitivity or resistance to a given therapy. Finally, last but not least, we may open up for the possibility to avoid unnecessary biopsy associated complications, minimize the potential risks of biopsy generated metastases and may also deliver diagnoses more timely and at a lower cost using FNAB based molecular profiling. These factors will be of outmost importance when the number of cancer patients increases worldwide.

References

1. Crawford S (2013) Is it time for a new paradigm for systemic cancer treatment? Lessons from a century of cancer chemotherapy. Front Pharmacol. 4:68.
2. Zhang YJ, Lichnan W, Zheng YO, Li XR (2013) Status quo and development trend of breat biopsy technology. Gland Surg 2: 15-24.
3. Franzén S, Zajicek J (1968) Aspiration biopsy in diagnosis of palpable lesions of the breast. Critical review of 3479 consecutive biopsies. Acta Radiol Ther Phys Biol 7: 241-262.
4. Hollerbach S, Juegensen C, Hocke M, Freund U, Nellman, et al. (2014) EUS-FNA: How to improve Biopsy Results? Z. Gastroenterol 52: 1081-1092.
5. Wolinski K, Strangierski A, Ruchala M (2016) Comparison of diagnostic yield of cor-needle and fine-needle aspiration biopsies of thyroid lesions: systematic review and meta-analysis. Eur Radiol.
6. Marks L, Young S, Natarajan S (2013) MRI-ultrasound fusion for guidance of targeted prostate biopsy. Curr Opin Urol 23: 43-50.
7. Povoski SP, Jimenez RE, Wang WP (2011) Ultrasound-guided diagnostic breast biopsy methodology: retrospective comparison of the 8-gauge vacuum-assisted biopsy approach versus the spring-loaded 14-gauge core biopsy approach. World J Surg Oncol. 9: 87.
8. Venkataraman S, Dialani V, Gilmore HC, Metha TS (2011) Stereotactic core biopsy: comparison of 11 gauge with 8 gauge vaccum assited breast biopsy. Eur J Radiol 81: 2613-2619.
9. Liebens F, Carly B, Cusumano P, van Beveren M, Beier B, et al. (2009) Breast cancer seeding associated with core needle biopsies: A systematic review. Maturitas 62: 113-123.
10. Imschweiler T, Haueisen, H, Kampmann G, Rageth, L, Seifert B, et al. (2014) MRI-guided vacuum-assisted breast biopsy: comparison with stereotactically guided and ultrasound-guided techniques. Euro Radiol 24: 128-135.
11. Shyamala K, Girish HC, Murgod S (2014) Risk of tumor cell seeding through biopsy and aspiration cytology. J Int Soc Prev Community Dent 4: 5-11.
12. Janssens JP, Rotenberg L, Sentis M, Motmans K, Schulz-Wendtland R (2006) Caution with microbiopsies of the breast: displaced cancer cells and ballistics. Eur J Cancer Prev 15: 471-473.
13. Pepe P, Aragona F (2013) Morbidity after transperineal prostate biopsy in 3000 patients undergoing 12 vs 18 vs more than 24 needle cores. Urology 81: 1142-1146.
14. Diaz LK, Wiley EL, Venta LA (1999) Are malignant cells displaced by large-gauge needle core biopsy of the breast? AJR Am J Roentgenol 173: 1303-1313.
15. Tyagi R, Dey P (2014) Needle tract seeding: an avoidable complication. Diagn Cytopathol 42: 636-640.
16. Koch M, Kienle P, Sauer P, Willeke F, Buhl K, et al. (2004) Hematogenous tumor cell dissemination during colonoscopy for colorectal cancer. Surg Endosc 18: 587-591.
17. Ladjevardi S, Auer G, Castro J, Ericsson C, Zetterberg A, et al. (2014) Prostate biopsy sampling causes hematogenous dissemination of epithelial cellular material. Dis Markers.
18. Bednarz-Knoll N, Alix-Panabières C, Pantel K (2011) Clinical relevance and biology of circulating tumor cells. Breast Cancer Res 13: 228.
19. Sosa MS, Bragado P, Aguirre-Ghiso JA (2014) Mechanisms of disseminated cancer cell dormancy: an awakening field. Nat Rev Cancer 14: 611-622.
20. Hüsemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, et al. (2008) Systemic spread is an early step in breast cancer. Cancer Cell 13: 58-68.
21. Klein CA (2011) Framework models of tumor dormancy from patient-derived observations. Curr Opin Genet Dev 21: 42-49.
22. Juratli MA, Sarimollaoglu M, Siegel ER, Nedosekin DA, Galanzha EI, et al. (2014) Real-time monitoring of circulating tumor cell release during tumor manipulation using in vivo photoacoustic and fluorescent flow cytometry. Head Neck 36:1207-1215.
23. Mathenge EG, Dean CA, Clements D, Vaghar-Kashani A, Photopoulos S, et al. (2014) Core needle biopsy of breast cancer tumors increases distant metastases in a mouse model. Neoplasia 16: 950-960.
24. Hobson J, Gummadidala P, Silverstrim B, Grier D, Bunn J, et al. (2013) Acute inflammation induced by the biopsy of mouse mammary tumors promotes the development of metastasis. Breast Cancer ResTreat. 139: 391-401.
25. Quail DF, Joyce JA (2013) Microenvironmental regulation of tumor progression and metastasis. Nat Med 19:1423-1437.
26. Luoto KR, Kumareswaran R, Bristow RG (2013) Tumor hypoxia as a driving force in genetic instability. Genome Integr 4: 5.
27. Mego M, Giordano A, De Giorgi U, Masuda H, Hsu L, et al. (2015) Circulating tumor cells in newly diagnosed inflammatory breast cancer. Breast Cancer Res 17: 2.
28. Huang XZ, Gao P, Sun JX, Song YX, Tsai CC, et al. (2015) Aspirin and nonsteroidal anti-inflammatory drugs after but not before diagnosis are

associated with improved breast cancer survival: a meta-analysis. Cancer Causes Control 26: 589-600.

29. Satyanarayana R, Parekh D (2014) Prevention and treatment of biopsy-related complications. Curr Urol Rep 15: 381.
30. Haas S, Park TW, Hahne JC, Fischer HP (2008) Influence of preoperative core biopsies on uPA/PAI-1 expression in breast cancer tissue. Virchows Arch 452: 277-283.
31. Arnold KM, Openaker LM, Flynn D, Sims-Moutada J (2015) Wound healing and cancer stem cells: Inflammation as a driver of treatment resistance in breast cancer. Cancer Growth and Metastasis 8: 1-13.
32. Weber M, Moebious P, Brünette-Herold M, Amann K, Preidl R (2015) Macrophage polarization change within the time between diagnostic biopsy and tumour resection in oral squamous cell carcinomas – an immunohistochmical study. Br J Cancer 113: 510-519.
33. Szalayova G, James TA, Rincon M (2015) A framework for the role of aute inflammation in tumor progression. Breast Cancer Res Treat 151: 235-238.
34. Zhang J, Liang Q, Lei Y, Yao M, Li L, et al. (2012) SOX4 induces epithelial-mesenchymal transition and contributes to breast cancer progression. Cancer Res. 72: 4597-4608.
35. Kronenwett U, Ploner A, Zetterberg A, Bergh J, Hall P, et al. (2006) Genomic instability and prognosis in breast carcinomas. Cancer Epidemiol Biomakers Prev. 15: 1630-1635.
36. Wiksell H, Schässburger KU, Janicijevic M, Leifland K, Löfgren L, et al. (2010) Prevention of tumour cell dissemination in diagnostic needle procedures. Br J Cancer 103: 1706-1709.
37. Pusztai L, Ayers M, Stec J, Clark E, Hess K, et al. (2003) Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably indentify routine prognostic markers and reveal large-scale molecular differences between estogen-negative and estrogen-positive tumors. Cancer Res 9: 2406-2415.
38. Ullal AV, Peterson V, Agasti SS, Tuang S, Juric D, et al. (2014) Cancer cell profiling by barcoding allows multiplexed protein analysis in fine-needle aspirates. Sci Transl Med 6(219): ra9.
39. Assarson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, et al. (2014) Homogenous 96-Plex PEA immunoassay exhibiting high sensitivity, specificity and excellent scalability. Plos One 9: e95192.