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The role of ELISA in the diagnosis of Alzheimer's disease

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Abstract. As the world population ages, Alzheimer's disease (AD) has emerged as a significant public health issue. The regulatory measures, clinical strategies, and even the scientific communications regarding AD all rely on the advancements in uncovering AD pathology, and the ability to recapitulate the disease-relevant players in a "gestalt" and clinically-applicable manner. Neurologists have sought to post mortem validation of AD pathologies (amyloid plaques and neurofibrillary tangles) in brain tissues; gradually, the weight is shifted to the detection of biological measures in the hope of diagnosing AD in its early phase before irreversible cognitive deterioration. Technologies that enable such early diagnosis or even prevention of AD, such as the enzyme-linked immunosorbent assay (ELISA), are emerging to obtain increasingly bigger roles in this field. Increasing the sensitivity and accuracy through enhancing the original ELISA with novel agents (nanoparticles as colorization agents etc) or improved protocols were the main approaches used. However, ELISA is also important as a lab staple, especially for the quantification of key AD biomolecules such as Ab42. ELISA connotates a more profound theme and future prospect of AD research: the entangling of biosensors development and the continued effort in deciphering of a more holistic map of AD.

Keywords: Alzheimer's disease; ELISA; Diagnosis.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease, and is the primary cause of age-related dementia. Its hallmarks have been identified as visible amyloid plaques and neurofibrillary tangles in brain histology when AD was first discovered more than a century ago. That was when Alois Alzheimer's report of this "peculiar, severe brain disease" barely stirred any response in the field of neuroscience, which, in stark contrast to now, where awareness for "Alzheimer's" and its mammoth impact drives countless studies to continue uncovering more of its underlying mechanism.

Indeed, immense progress has been made since the acknowledgement of amyloid-beta and tau as key players in AD in correspondence to the AD pathology identified post mortem, including the invention of drugs e.g. Lecanemab aimed at dismantling aggregated amyloid and phosphorylated-tau proteins in diagnosed AD patients. However, the proportion of cognitive degeneration unavoidable by drugs alone, the complexity of underlying biological pathways connotated by the known "corner of the iceberg", and the fact that AD in its early stages can resemble many other neurological disorders urges on the search for early recognition approaches.

Biosensing is thus intimately linked to the early diagnosis of AD. Many types of household biosensors were already utilized for detection of AD-biomarkers, with ELISA being a prominent example. However, the use of ELISA for AD remains entangled with inadequacy of sensitivity and certainty. For example, Ab42, the most susceptible variant among amyloid proteins to aggregation while also a convenient target for biosensing approaches, was proposed as one of the initial targets for biosensing [1]. Multiple ELISA protocols have been developed and validated for the routine detection of Ab42 in samples, smoothing the process of experimental research in AD pathology. However, while measuring cerebrospinal fluid (CSF) samples is convenient, it's also invasive and can potentially induce negative after-effects on patients, while the fluctuation of Ab42 level in the plasma of early phase AD patients renders the endeavor to hone current ELISA techniques towards further sensitivity not discontinued. Toward the same cause, novel biomarkers such as tau-related enzymes, or, more

recently, the series of interconnected cytokines inspired by the discovery of abnormal microglia activity and overall neuroinflammatory response linked to AD were newer attempts at higher diagnostic accuracy [2]. ELISA is emerging as both a beam of hope to rejuvenate many potential biomarkers, and a recurring theme in cutting-edge studies of AD. This renders it a perfect medium to obtain both the what and how of the current pathological portrait of AD. It also points at a future of multiplex biosensing, which yields the possibility of personalized diagnostic, or even prognostic, multi-marker profile for AD.

2. Application of ELISA for the diagnosis of AD

2.1. Improving the sensitivity of ELISA

There have been many attempts at improving the sensitivity of ELISA in the past decade. Hashimoto et al [3] created a novel ELISA assay by renovating the sandwich ELISA system from Wako Chemicals GmbH (Neuss, Germany) through eight-times dilution of the original buffer by RIPA buffer and substituting the original colorimetric agent with Amplex UltraRed, which dampened the noise level and overall increased the sensitivity of ELISA by around 10 times. They were able to achieve intra-neuron detection of Ab, which was unprecedented. This is to serve the recent evidence suggesting that intracellular level of Ab, while being significantly lower and harder to detect than the knowingly important extracellular measurements, could also be of importance. Indeed, the results yielded by the novel ELISA are in accordance with the confirmed AD neuropathology: hippocampal pyramidal cells are impacted more than cerebellar purkinje cells. This adds intracellular Ab42 to the potential repertoire of diagnostic parameters. This study also combined ELISA with another novel technique-laser capture microdissection, highlighting the hybridizing and freeform trend in detecting AD early.

Novel ELISAs were also developed against the other AD hallmark---tau protein. Similar to the alteration from extracellular to intracellular detection, these new assays were mainly aimed at exploring blood plasma as a non-invasive alternative to CSF sample, which is the only plausible option if using conventional ELISA, bridging the gap between diagnostic detection and the optimal sampling method. Combinations of designated, high-affinity antibodies and digital output approach to allow both the lowering of LOD and the elongation of dynamic range. The minutia of the protocols, such as incubation conditions and times between tau and antibody and immobilizing agent and tau are optimized through trials [4]. The latter permits tau-targeting ELISA platform to be flexible for a variety of biological samples, which is especially important for the variety of relatively unknown phosphorylated tau species (p-tau-231, -181 and 235). Such trend of ELISA development can provide valuable additions to the current AD diagnostic repertoire, such as MRI or cognitive screening, which are either too expensive or not timely enough for robust treatment [4]. Although, the complex pathological landscape hints at the large set of antibodies needed to pinpoint each tau species.

One example would be ELISA targeting p-tau231 [5], which reduces the tau-microtubule affinity, as well as promoting the phosphorylation of other tau residues and subsequently loss of function. It is perhaps more pivotal than other p-tau species such as p-tau181 because it can potentially help distinguish AD from other dementias, such as frontotemporal dementia, vascular dementia, and dementia with Lewy bodies. Santos et al tested the potential of p-tau231 as a biomarker in a therapeutic scope. They selected the clinical testing cohort with care: as opposed to the common “known AD patients” vs “healthy control” which yields significant yet artificial contrast, this study utilizes a complex amalgamation of patients with different types of dementia as well as AD to mimic the high noise level in actual diagnostic setting. This study also has its focus on validating the ELISA protocol itself, such as evaluating the impact of thaw-freeze cycles, incubating temperature etc. The result does confirm p-tau231 as a potential biomarker, but limits of the study include significant inter-assay variance between this and the other validated p-tau assays. The situation against tau proteins is a synecdoche of ELISA in AD: it highlights both the significance of ELISA in unlocking many of the pathological players as validated biomarkers, and emphasizes how this ‘paraphyletic’ evolution of

ELISA protocols face a lack of standardization. Further testing is certainly required before such modified, AD-specific ELISAs can enter the clinical setting.

2.2. ELISA as a staple in clinical research on AD

Nevertheless, the major occurrence of ELISA is still to assist the further uncovering of AD pathology. In a very recent publishing, Zhao et al. uncovered that b2M (beta2-microglobulin), a previously under-investigated pathological factor, coaggregates and plays important role in Ab toxicity in AD, and most steps testifying the impact that the absence or over-abundance that b2M plays are conducting using ELISA, showing its handiness [6]. b2M is a component of major histocompatibility complex class1 (or human leukocyte antigen (HLA) in humans), the haplotypes of which are linked, like many other immune-related genes, to increased risk of AD as identified by GWAS. It exists as a monomer under normal physiological conditions, yet has been found to assemble into amyloid oligomers under pathologies such as kidney diseases. The heightened level of b2M in CSF in patients with AD and other dementia and the upregulation of B2m expression in microglia of FAD mice also suggest a potentially important role that b2M plays in AD. This is important because Ab aggregation alone is shown to be inadequate to trigger AD cascade, and tackling other players such as b2M (over 50 risk factors have been discovered with unknown mechanism) can shed light on novel AD treatments. Staining of both human and mouse brain shows that >80% of Ab plaques stained positive for b2M, suggesting coaggregation, which is also confirmed by ELISA. AAV-b2M, or pure b2M, injection markedly increased both soluble and insoluble Ab42 level in 4 months' time as detected by ELISA, and in an MHC class1 independent manner, contending that there is indeed a noncanonical role of b2M in AD pathology. Genetic ablation of B2m in mice significantly reduced Ab42 deposition in a gene dosage-dependent way, and likely casting its impact through Ab clearance rather than the APP processing pathway as the levels of key players in the latter remained unperturbed. B2m-deleted mice also possess better cognitive abilities as demonstrated through spatial and associative memory tests. More intriguingly, b2M is shown to be able to cross BBB. By parabiotic surgery, the authors were able to conjoin a 5xFAD and a B2m-/- mouse and examine the impact of them exchanging blood. The blood from B2m-/- mouse is shown to markedly reduce b2M level and amyloid deposition in brain of 5xFAD mouse (as shown by both immunoblotting and ELISA) while that of 5xFAD's induced b2M-specific antibody in B2m -/-. This exhibits the impact of peripheral b2M clearance as curiously equivalent to B2m deletion, while the detailed mechanism remains unsure of.

Also aiming to uncover the pathology of AD from the peripheral end, Xia et al. performed fecal microbiota transplantation (FMT) of healthy donors and AD patients respectively to 2 different cohorts of Thy1-C/EBPb transgenic mice (mice that mimic aged animals, as C/EBPb level is associated with AD-triggered microglia activity and neoropathologies) [7]. AD pathologies such as Ab and tau developed in the Thy1-C/EBPb mice that received recolonization as shown by IF staining with AD fecal samples but not those with HC samples nor the wild-type mice, in association with AEP pathway upregulation, microglia activation, and cognitive defects. ELISA was able to pinpointedly recognize the increase of Ab42 rather than Ab40 in the AD group. rRNA analysis, simultaneously, showed the difference being an abundance of B. fragilis in the AD group. Treatment by the metabolites of these bacteria also induce the same effect; conversely, primary rat microglia activation assay confirmed the stimulating effect of bacterial PUFA metabolites, 12-HHTrE and PGE2, on microglia, which yielded similar response to the neuroinflammation observed in AD. Metabolomic analysis revealed that, in accordance, PUFA metabolism is enhanced in mice after AD-FMT. The result is crucial as microglia is one of the most key flags for neuronal degeneration, and gut microbiota is linked to microglia through the former's metabolites across the gut-brain axis. The availability of a variety of ELISA kits allows the convenient testification of a hypothetical disease-relevant factor.

The trace of ELISA can also be found when testing for comparatively pleiotropic disease phenotypes. As shown in Figure 1, Warfield et al. linked pTau pathology in lateral parabrachial (LPB) neurons, especially those expressing dynorphin, to the common phase delay and sundowning shown by 20-25%

AD patients [8]. This is important because sundowning is one of the most poorly understood circadian-related disturbances. SCN is the master mediator of circadian rhythms, yet the usual defect that leads to circadian dysfunction---loss of SCNvip---is unavailable in AD, suggesting that the defect likely lies in the inputting end to SCN. ELISA was used in this study to validate the antibodies for immunohistochemistry against Ab and pTau. This exhibits the versatility of ELISA in AD research.

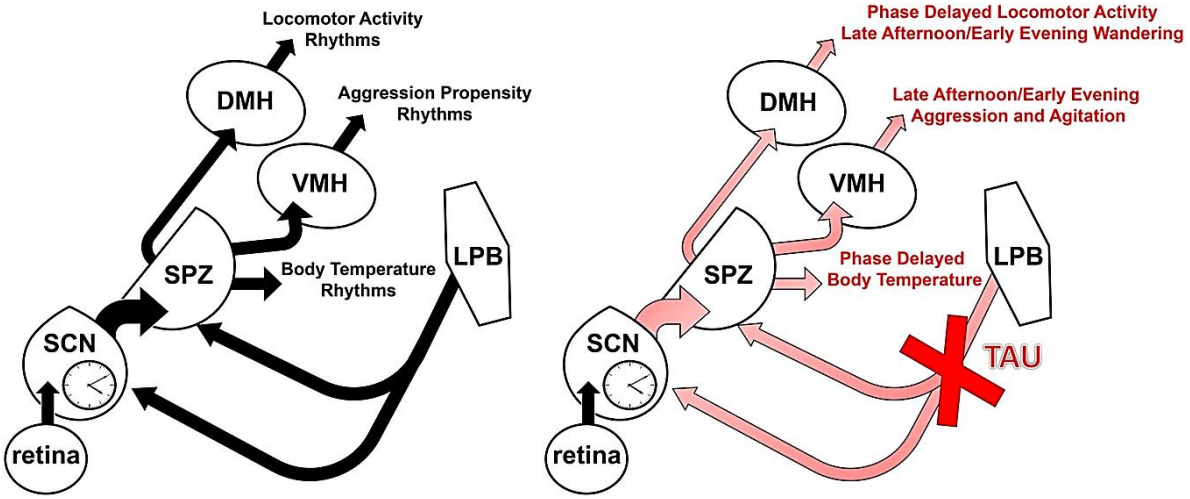

Figure 1. The neural circuit underlying sundowning-related symptoms in AD [8].

2.3. The combination of ELISA and novel biotechnologies

Organoids, regarded as one of the most potentiated tools for neurological research due to their ability to recapitulate human pathology better than animal models, were starting to be widely used in AD research. ELISA's versatility can complement this novel modelling method and accelerate clinical research and application. Jorfi et al. created a 3D human neuroimmune axis model. The infiltration number of T cells is relatively and significantly higher in the AD than control cultures, which led to microglia activation and neuroinflammation (ER stress) which is highly relevant to neurodegeneration [9]. Physical neuronal damage was observed as rapid as <72h after CD8+ T cell infiltration in the AD cultures and in the presence of microglial cells only, corroborating the important role of T cell infiltration in exacerbating damage through overstimulation of microglia as a mechanism underlying AD. ELISA revealed significant decrease in Tuj1 (marker for differentiated neurons) and upregulation in Interferon (IFN) pathways e.g. INF-b in AD cultures with microglia and CD8+ T cells.

Zhao et al. developed cerebral organoids using iPSCs with either APOE4 or APOE3 from both AD patients and healthy controls [10]. It was shown that AD patient-derived organoids with APOE4 genotype developed greater extent of apoptosis and decreased synaptic integrity. Curiously, APOE4 aggravates tau pathology in both healthy subject-derived and AD patient-derived organoids, and isogenic conversion from APOE4 to APOE3 ameliorates APOE4-related pathology in AD models. The use of ELISA allowed the quantification of Ab40 and Ab42, which has been found independent of APOE4 and APP processing, pointing the causal factor to Ab clearance mechanisms. The fluidity of ELISA allowed it to also detect apoE and p-tau at key time points and upon conversion from APOE4 to APOE3.

3. Conclusion

The trek of AD continues, thanks to the advancements of technologies that allow us to both clarify the resolution of pathological changes to single cell level, and to gradually uncover the overall landscape and grasp the new lights for AD diagnosis and treatment. Recent discoveries regarding the variance of transcriptomic and epigenomic states of key players such as microglia as AD progresses offer novel treatment ideas, and as a first step of which help with the timely diagnosis and guide the innovation of biosensors. More explicitly, big datasets of pan-nuclei AD transcriptome show the

ubiquity of AD pathologies among different cell types, casting away the web of uncertainty and rendering the challenge of actually translating such information into clinical strategies, such as pinning down the diagnosis as early as possible using these newly-discovered biomarkers, the priority. Striving to unlock new biomarkers, the LOD of AD ELISAs are being brought down to picomolar and even femtomolar levels, although still not in real physiological conditions. Thus, the intimate umbilical cord between cutting-edge AD research, where ELISA is used as a staple, and the future of ELISA as a synecdoche of all biosensor's hints at the need for yet further researches in search of potential biomarkers, after which biosensors can potentially sublimate the meaning of these markers by marrying diagnostic applications with optimal sensitivity and selectivity.

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