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Abstract

A survey amongst the participants of the Paraneoplastic Neurological Antibody Scheme, registered with United Kingdom National External Quality Assessment Service (UK NEQAS), across the UK, continental Europe and non-European countries examined various factors involved in the laboratory diagnostic methodologies, timely provision of results and a snapshot of External Quality Assessment (EQA) performance to ascertain the level of harmonisation amongst participating laboratories. Despite variations in some aspects of the analytical methods there appears to be a good agreement in the outcome of the results as demonstrated by the EQA performance.

Introduction

Laboratory contribution to clinical diagnosis is an essential part of patient care. In order to accurately diagnose, treat and advise patients, physicians rely on timely laboratory data that remains consistent regardless of its origin and most will take the quality (accuracy, reproducibility, clinical relevance) of the result for granted. Most physicians rightly assume that quality is assured as a routine part of the work of the laboratory and would not expect that different versions of tests would produce different results on the same sample, or that exactly the same test on the same sample might produce different results in different places. Laboratories and their suppliers strive to achieve this by monitoring and standardizing test methodologies with the aid of robust internal and external quality control. Where standardization (same result in same units on the same sample, everywhere) is not possible, we aim for harmonization of reporting outcomes (all positive and negative results match, irrespective of units). Despite such an ethos, laboratory results on the same patient sample can vary due to rapid development in the diagnostic service or methodology, or the pressures of increasing workload. From time to time, in order to continue with the ethos of improving patient care and outcome, it is essential to examine procedures; where a problem is intractable and important we may need to develop guidelines/best practice advice to attain harmonization.

We sought to examine how effectively we had achieved the above goals in a specialized neurological test for the Paraneoplastic neurological syndromes (PNS) that are associated with paraneoplastic neurological anti-neuronal antibodies (PNA). Paraneoplastic neurological syndromes are autoimmune disorders where the remote immunological effects are triggered by the presence of a (often occult) tumor. This autoimmune response results in neurological signs due to neuronal damage or dysfunction. The first credible evidence for such malignancy-associated autoimmunity, misdirected against neurological tissue, was provided by Posner in 1985 [1]. PNA are an invaluable early and precise diagnostic marker of rare debilitating neurological disorders. Furthermore, these antibodies do alert the clinicians of possible existence and location of underlying malignancy. Consequently, the early diagnosis of PNS can often lead to the discovery and effective treatment of the underlying malignancy, and is also a crucial step in the management of the PNS [2].

In the early years, detection of PNA suffered from variability due to non-standard procedures, often developed and validated in-house by research groups [3-7]. As these become adopted widely, or were translated into commercial versions of the original assay or new look-alike variants, we saw increasing disparities in inter-laboratory comparisons, thus prompting the development of the first guideline for detection and classification of paraneoplastic anti-neuronal specific antibodies [8]. This guideline was supposed to provide greater harmonization of use and reporting, but covered only three antibodies

of subjects with PNA and neurological symptoms may not have a detectable neoplasm.

These anomalies created a need for further standardization of diagnostic criteria and classification of PNS. This was addressed by a study supported by the European Union to define standards for the diagnosis and classification of PNS [10]. However, the authors noted and raised concerns that detection methodologies for neuronal antibodies were not widely standardised and to our knowledge there has been no significant improvement since then. As a pre-requisite to achieve clinically useful standardization, it is essential to have inter-laboratory monitoring via independent EQA Schemes.

Due to diversity in the clinical syndromes and autoimmune neurological response, screening for a range of neuronal antibodies is now thought more effective than testing for specific PNA individually. However, there are considerable difficulties in obtaining enough positive control material to cover all the rare specificities. There was a clear need and role for an EQA scheme in this area.

In 2010, the challenge of developing external quality assessment was taken on by United Kingdom National External Quality Assessment Service for Immunology, Immunochemistry & Allergy (UK NEQAS IIA) at the behest of its independent steering committee. UK NEQAS IIA introduced a pilot scheme for paraneoplastic neurological antibodies, sending out two samples every two months. Five years later, this scheme has grown to over a 100 international participants.

Identified with ease antibodies		
Antibody	**Neurological disorder(s)**	**Most frequent tumour(s)**
Hu (ANNA1)	Paraneoplastic cerebellar degeneration, paraneoplastic encephalomyelitis, sensory neuropathy	Small cell lung carcinoma
Yo (PCA-1)	paraneoplastic cerebellar degeneration	Ovary, breast
Ri (ANNA2)	opsoclonus/myclonus, paraneoplastic cerebellar degeneration, brainstem encephalomyelitis	Breast, small cell lung carcinoma, gynaecological tumours
Difficult specificities		
Ma2 (Ta)	brainstem encephalomyelitis, limbic encephalopathy	Testicular cancer
CV2/CRMP5	paraneoplastic encephalomyelitis / sensory neuropathy	Small cell lung carcinoma, thymoma
Amphiphysin	Stiff person syndrome, paraneoplastic encephalomyelitis	Breast cancer, small cell lung carcinoma
Tr (PCA-Tr)	Paraneoplastic cerebellar degeneration	Hodgkin's lymphoma

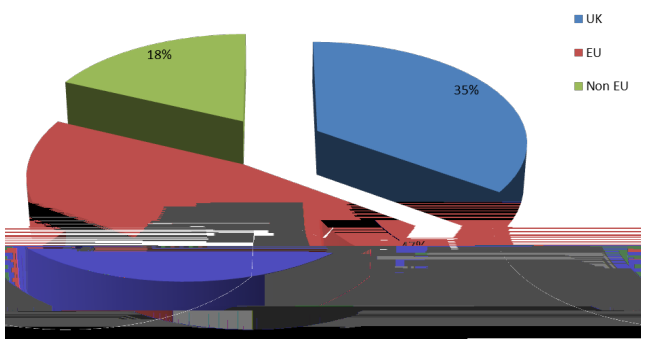

Figure 1: Location of laboratories.

Repertoire

Most services ofered the entire major antibody profle (Hu, Ri, Yo, CV2, Ma2 and amphiphysin) associated with PNS (between 89 to 96% for each specifcity).

96% ofered the 3 easily identifable antibodies (Hu, Ri and Yo) and 89% also ofered (CV2, Ma2 and amphiphysin).

Specialised and antibody detection of poorly characterised specifcities, i.e. anti-Tr, appears to be confned to fewer centres (63%; Table 3a).

Turnaround time

The turnaround time was variable: for negative screening 87% reported within14 days but positive identifcation was only achieved in 14 days for 66%. 23% of laboratories report a positive result between 15 to 28 days, but 11% take between 29 and 45 days (Table 3b).

Screening methodology

There is a clear consensus (90%) for the use of primate cerebellum as an initial screen for the detection of PNA (Table 4a). A few labs still use rat tissue (6%) or other assays (such as immunoblot) for primary screening (4%). Most screening tissue substrates were supplied by commercial manufacturers and were C.E marked.

Screening dilution

Blood: These are variable, and require local validation of sensitivity. They ranged from 1/10 to 1/100 but 40% used 1/50, and 27%; 1/10 (Table 4b).

CSF: Only 64% of participants ofered CSF screening. 50% advocate testing neat CSF but a few (14%) utilise a range of dilutions (from 1/5 to 1/20). The remaining 36% had no provision for CSF analysis for PNA (Table 4c).

However, it was very interesting and surprising to learn that 21% (10/48) of the contributors combined different samples together for screening purpose (Table 4d).

of positive screens

Following the frst serum or CSF incubation step, the bound human immunoglobulins were detected by fuorescence labelled anti-human immunoglobulin (96%) and only two participants resort to other means (Table 4e). In the event the screen was positive; the identity of the specifc antigen was confrmed by commercial immunoblotting procedures by most (85%; Table 4f).

			% (n)
		Commercial immunoblots	40 (85)
(f)	Confirmatory test -	In house	1 (2)
		Referral	6 (13)