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Report

Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs)

Geneva Switzerland
21-22 September 1999

WORLD HEALTH ORGANIZATION
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REPORT

1.1 Background

The need for Reference Reagents and Reference Panels to facilitate development and comparison of various diagnostic approaches and assay systems for diagnosis of TSEs, including CJD in humans, bovine spongiform encephalopathy and scrapie of sheep and goats was recognized at a WHO Consultation on Diagnostic Procedures for Transmissible Spongiform Encephalopathies (TSEs) held in March 1999.

Although considerable progress in the laboratory tests was reported, the predictive value of these assays in terms of TSE infectivity has not yet been established and correlation with bioassays in animal models will be required. Several technical questions concerning sensitivity, specificity, application to different tissues etc. will have to be answered in order to compare assays and results obtained by different groups. Therefore, the WHO Secretariat was requested to form a WHO Working Group on International Reference Materials for the Diagnosis and Study of TSEs as a matter of urgency.

The urgent need to develop a panel of International Reference Materials has also been recognized at international meetings organized by the European Agency for the Evaluation of Medicinal Products (January 1999) and the Center for Biologics Evaluation and Research (CBER) and the Center for Devices and Radiological Health (CDRH) of the United States Food and Drug Administration (FDA) (September 1999). Those Agencies concurred that detection of markers for TSEs was an urgent priority and strongly supported the development of the WHO project.

Internationally agreed-upon reference materials will be essential to validate diagnostic procedures which could be used to exclude potentially infectious donated blood, organs and tissues, to identify people who might benefit from therapeutic interventions, and in the validation of pharmaceutical manufacturing processes to prove the potential capacity of these processes to remove transmissible agents.

The first Meeting of the Working Group was convened at WHO Headquarters, Geneva, on 21-22 September 1999. The terms of reference of the Working Group and list of participants are attached as Annexes 1, 2 of this Report.

1.2 Conclusions and Recommendations

The Working Group discussed topics enumerated as goals in the Terms of Reference. Several conclusions were reached and recommendations offered:

1.2.1 Sources of candidate human TSE Biological Reference Materials were identified as being of high priority. Those include preparing at least 100 gram-samples of cerebral cortical grey matter from:

- new variant CJD, one case

- the most common form of sporadic CJD (with type-1 protease-resistant prion protein [PrP^{Sc}] in a patient homozygous for methionine at codon 129 of the *PRNP* gene), three cases

- sporadic CJD (with type-2 PrP^{Sc} in a patient homozygous for methionine at codon 129 of the *PRNP* gene), one case

- control (no clinical nor pathological evidence of neurological diseases), one case

Tissues will be provided by Drs Gambetti, Budka and Ironside. All preparations should be grey matter enriched. Histopathological evaluations will be performed by the providers. Relevant epidemiological, clinical and neuropathological information will be available for each case although the identity of each patient will be confidential and not disclosed to participants in the development of the Biological Reference Materials.

Each sample will be mechanically homogenized at 4(C in 0.32 M sucrose to obtain a 10% (wt/vol) brain suspension. The brain suspension will be clarified by gravity for 10 minutes at 4(C and the supernatants transferred to a continuously-stirred sterile vessel from which aliquots of 0.5 ml will be distributed into sterile plastic screw-cap vials. A full protocol will be developed by Dr Hope.

Samples will be prepared and stored at NIBSC (UK) which will conduct preliminary evaluation for bacterial sterility, protein and nucleic acid contents, and presence of PrP^{Sc}. NIBSC will provide the documentation and distribute the materials.

The master protocol for a WHO Collaborative Study with these Biological Reference Materials will be prepared by Dr Minor and circulated for comments. Quantitative Western Blot, other PrP^{Sc} assays and infectivity assays will be included in the master protocol. Qualified participants will be solicited through invitations issued by the WHO.

1.2.2 The Working Group concluded that development of reference materials for BSE and scrapie would be more appropriately done in collaboration with the Organization International des Epizooties and relevant national agricultural and veterinary authorities. Attention was drawn to a major European Union study on the titration of BSE infectivity which is currently in progress. Representatives will be invited to the next meeting of the Working Group to explore these possibilities. The diversity of scrapie strains and genetic differences in host susceptibility will have to be considered in selecting scrapie Biological Reference Materials. The Working Group also decided that, at the moment, it does not seem necessary to provide reference rodent-adapted strains of TSE agent, several of which can be easily obtained.

1.2.3 The Working Group also recommended that, before starting the collection of blood from patients and animals with different forms of TSE, it will be important to reach consensus on methods of collection of blood and separation of plasma since some of these procedures may affect the detection of infectivity. Scientists involved in the collection of blood and separation of plasma will be invited to the next meeting of the Working Group to address this issue. Dr Rohwer was asked to prepare a proposal to present to the Working Group then.

1.2.4 Although the value of biological reference antibodies for improved immunodiagnostics and for detection of PrP is apparent, the Working Group saw no current need to develop such antibodies under the auspicies of WHO.

1.2.5 The Working Group felt that the WHO document Global Surveillance, Diagnosis and Therapy of Human Transmissible Spongiform Encephalopathies: Report of a WHO Consultation, Geneva, Switzerland, 9-11 February 1998, WHO/EMC/ZDI/98.11 should provide sufficient information to ensure consistent clinical and histopathological diagnosis of human TSEs.

1.2.6 The participants agreed that a consistent terminology for types and subtypes of human PrP^{Sc} would be desirable. The Working Group encouraged the current efforts of a project of the European Union, coordinated by Dr Budka, to harmonize this terminology and suggested that it would be helpful to enlarge this effort by distributing samples for study outside the European Union under the auspices of the WHO as a WHO Collaborative Study of Human PrP^{Sc} types.

1.2.7 The Working Group agreed that it will be extremely important to titrate infectivity of each candidate Biological Reference Materials in a variety of conventional and transgenic rodents and in primates. They emphasized that this will be impossible unless additional funding is secured.

1.2.8 The Working Group agreed to distribute the samples of the candidate Biological Reference Materials to any qualified laboratory requesting them to compare sensitivity and specificity of various immunoassays for the detection and quantification of PrP^{Sc}. Immunoassays can be performed by laboratories participating in the WHO Collaborative Studies without additional funding.

1.2.9 The Working Group also agreed to prepare panels of replicate coded randomised dilutions of one or more of the candidate Biological Reference Materials including one or more controls for the comparison of analytical sensitivity in detecting PrP^{Sc} with different assays. Great attention should be paid to differentiate between PrP^{c} and PrP^{Sc}; that will eventually require the inclusion of a larger number of control uninfected brain samples, including those from patients with other neurological diseases. The development of the protocol and preparation of the samples will be responsibility of CBER (FDA, USA). A draft protocol will be developed by Dr Asher and circulated for comments.

1.2.10 Members of the Working Group potentially participating in the WHO Collaborative Study agreed to estimate the funding that would be required for them to perform the infectivity titrations in various animals. These estimates will be provided to the WHO Secretariat before 1 January 2000.

1.2.11 The Working Group reviewed and agreed to the attached Terms of Reference.

1.2.12 Proposed dates for next Working Group Meeting: 8-9 May 2000

ANNEX 1

WHO WORKING GROUP ON INTERNATIONAL REFERENCE MATERIALS FOR DIAGNOSIS AND STUDY OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSEs)

TERMS OF REFERENCE

Working Group Objectives

To develop internationally agreed-upon Reference Materials for the assessment and validation of assay systems to be applied in diagnostic procedures of TSEs. To identify needs for Reference Reagents and Reference Panels and to develop a WHO Repository to facilitate development of improved diagnostic tests based on available research methods.

Specific Goals

- Identification of the priorities for development of Biological Reference Materials relevant to public health for the objectives indicated above
- Development of a WHO Repository for positive and control materials derived from humans and animals with TSEs:
  - Selection and characterization of appropriate candidate materials
  - Development of protocols for WHO Collaborative Studies
- Development of additional internationally agreed-upon parameters for classification of human TSEs:
  - Harmonization of procedures and reagents used for the classification and nomenclature of PrP^{Sc} typing in human TSE cases
- Consideration of issues concerning the appropriate uses of the Biological Reference Materials
- Estimation of funding needs for the development of the project
- Follow-up of scientific developments with potential public health impact in the field

Membership

Members of the core group are drawn from academic and government research institutions and WHO Collaborating Centres. The Membership of the Working Group may be extended as appropriate to ensure that all the relevant scientific input is provided to the activities of the group.

Mechanism for Evaluation of Activities

Data produced by WHO Collaborative studies will be evaluated by the Working Group. Transparency of the activities will be ensured through WHO Consultations and adequate interaction with the world-wide scientific community. WHO will disseminate the information through the Internet at the following address:

http://www.who.int/technology/biologicals/biological.htlm

The outcome of the activities of the Working Group will be submitted to the Expert Committee on Biological Standardization, the WHO Committee responsible for the establishment of the International Biological Reference Preparations and Guidelines, for suggestions and endorsement.

ANNEX 2

WHO WORKING GROUP ON INTERNATIONAL REFERENCE MATERIALS FOR DIAGNOSIS AND STUDY OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSEs)
WHO Headquarters, Geneva
21-22 September 1999

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