Thalidomide Embryopathy

Report of a meeting of experts

World Health Organization (WHO)
Geneva, 2014

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Opening

The Meeting of Experts on Thalidomide Embryopathy was held at the headquarters of the World Health Organization (WHO) in Geneva .The meeting was opened by Mr Sten Olson of the Uppsala Monitoring Centre (UMC), Sweden, which carries out the international drug monitoring programme on behalf of WHO. Mr Olsson welcomed participants to the meeting and took the chair and introduced the agenda.

Dr Shanthi Pal of WHO headquarters added her welcome on behalf of WHO and emphasized the importance of the work being done by the experts present. Dr Pal said that, if the conference participants felt that their discussions should be taken further within WHO, it would be possible to share their views with the WHO Advisory Committee on Safety of Medicinal Products.

Participants then introduced themselves around the table. The list of participants is attached as Annex 1.

All participants in the meeting had received in advance a review of diagnostic criteria for thalidomide embryopathy prepared by St George's Hospital, University of London, United Kingdom. The review, which is attached as Annex 3, describes the main features of thalidomide embryopathy, shows the extent to which genetic analysis can help to differentiate between the effects of thalidomide and similar types of damage of known genetic origin, and provides a review of the relevant scientific literature.

Causation and probability

Dr Ralph Edwards of UMC introduced the issue of causation in pharmacovigilance, pointing out that a series of questions need to be asked, namely: "Can a drug cause a specific effect? Does it do so? And did it do so in a specific person?" He stressed the importance of being as sure as one can be about the premise and the data.

Dr Edwards referred to Austin Bradford Hill's work on causation in the 1930s which pointed to nine elements that need to be considered: temporality (did the cause precede the effect?), consistency (in similar circumstances is there the same effect?), strength (how often is this relationship seen in nature?), specificity (if you specify a set of circumstances, do you get the relationship?), a dose−response relationship (with a bigger dose, is there a bigger response?), experimental support (in controlled situations, does the same thing happen?), plausible mechanisms for effect, coherence (do other tests and data support the causation?), and analogy (with other similar causes and effects). The first five are elements that can be seen in human beings who have taken a particular medicine.

It was pointed out that the meeting would try to identify a core set of effects that all would agree can be seen with thalidomide. Dr Edwards drew attention, however, to a

number of challenges that need to be faced in doing so. Definite, or at least probable, effects may be identified, but there are also possible effects (less frequently seen, or commonly caused by other diseases), and there are other medicines that may have effects that overlap with those resulting from the use of thalidomide.

In pharmacovigilance, exposure to a drug is an absolutely necessary factor in considering causation. With thalidomide, however, there is the additional question as to whether the timing of exposure has an effect. Thus the meeting would need to consider whether thalidomide has to be taken at a particular time during gestation in order to have a harmful effect, and whether it is known how many women took thalidomide without any teratogenic effect? These would be important issues to resolve since the search for causality is based on probabilities, and other competing probabilities. As far as research findings are relevant to causation, one should ask whether continuing research into the effects of thalidomide is really being conducted with thalidomide or whether researchers are using newer experimental analogues and making analogies to thalidomide.

Pharmacovigilance operates with the concepts of "necessary" and "sufficient" cause. Only when there is necessary cause can it be inferred with certainty that a drug causes a specific problem every time it is used. If not, then one must always take the possibility of other causes into account and the competing causal probabilities for each of the effects of thalidomide should be assessed. Meeting participants were reminded of some of the relevant pharmacovigilance classification terms used in relation to causality – i.e. is the cause necessary, sufficient, contributory or secondary? For instance, "if D does not happen, E will never happen" indicates a necessary cause or condition for causality, while "if D or Z happens, E will happen" indicates a sufficient cause or condition. "If D happens, E may happen, but only with Z" indicates a contributory cause or condition, and "if D happens Z may happen and then E happens" indicates a secondary cause.

Stressing the need to define the thalidomide syndrome, Prof Edwards said that one should aim to reach a high (80%) probability of thalidomide causation. Even then, one would have to accept that there may be other effects of thalidomide that are not being diagnosed and there may be effects not due to thalidomide that overlap with the thalidomide effects. He asked participants to consider what would be the effects with at least 80% causality, and what would be the effects with a likelihood of causality of less than 50%. The question was raised as to what extent "reverse causality" (i.e. if this is the effect it must be related to thalidomide) had an influence on attitudes to the drug.

In discussion, it was noted that for thalidomide there was not always a dose–response relationship. In some cases severe damage was found after taking only one tablet on one occasion.

Classification of limb defects and the incidence of limb reduction defects

Dr Anna Latos-Bielenska of the Department of Medical Genetics of the University of Medical Sciences in Poznan, Poland, described the classification of limb defects both in Poland and in Europe more generally. The University of Medical Sciences houses the Polish Registry of Congenital Malformations (PRCM) which was established in 1998. Since 2001 PCRM has been a member of EUROCAT, which is a network of population-based registries for epidemiological surveillance of congenital anomalies, covering 1.7 million births in 21 countries of Europe.

The Department of Medical Genetics includes both the PCRM and a programme for investigation of the molecular basis of eye diseases, urinary system malformations, mental disability and limb malformations. As a member of the EUROCAT network, the department is contributing to research aimed at 1) developing a European reproductive pharmacovigilance system aimed at ensuring the safety of drugs used in pregnancy, 2) quantifying the risk of congenital anomalies related to antiepileptics, insulin analogues, anti-asthmatics and antidepressants, and 3) developing a framework for evaluating the efficacy of pregnancy-related drug safety measures. Four projects on limb malformations are currently in progress in Poznan – three involving the identification of novel genes or novel copy-number variations linked to limb malformations, and one on fetal vascular accident (antenatal thrombosis) as a possible cause of unilateral limb reduction.

Dr Latos-Bielenska reported that Polish statistics show that congenital limb malformations occur at the rate of 1 in 500 to 1 in 1000 live births, with the malformations occurring bilaterally in around half of the cases. Up to 18% of the children die before the age of 6 years, usually as a result of their malformations. These children represent a very heterogeneous group. In about 60−70% of cases, the limb defect is an isolated trait, while in the remaining 30−40% it is part of a multiple congenital anomaly syndrome. More than 500 multiple malformations or associations with limb defect have been described to date in Poland, with at least 50% having genetic causes.

With regard to classification, terminal limb deficiency defects are classified as complete (amelia) or partial (meromelia). A partial limb deficiency defect may involve an intermediary segment with a normal distal limb (intercalary) or may have no normal components beyond the most proximal level of malformation (terminal). The malformation may extend across the whole width of the limb (transverse) or involve only one segment (longitudinal) which can be further subdivided into pre-axial, central or post-axial. Dr Latos-Bielenska pointed out that the traditional ISO/ISPO classification of congenital limb deficiency (published by the International Society for Orthotics and International Society for Prosthetics and Orthotics) has drawbacks in that it classifies all limb deficiencies as either transverse or longitudinal. She then showed descriptions of limb deficiencies according to the ISO/ISPO and embryological classifications.

The meeting was shown illustrations of limb reduction defects classified as follows:

Transverse deficiencies

Congenital transverse deficiency is defined according to the last remaining bone segment. A short below-the-elbow amputation is the most common transverse deficiency, and is rarely associated with other anomalies.

- Phocomelia is a longitudinal failure of formation with an absent intervening segment of the extremity (intercalary aplasia).
- Brachydactyly

This refers to shortening of the hands and/or feet due to missing, deformed or shortened bones. It may occur as an isolated trait or as part of a syndrome. According to the pattern of skeletal involvement, the isolated brachydactyly forms are categorized in groups with several subgroups (with considerable overlap).

- Radial deficiency

One of the classic anomalies associated with systemic conditions is radial deficiency, which affects the preaxial border of the limb. The degree of preaxial deficiency can range from mild thumb hypoplasia to complete absence of the radius. Irrespective of the degree of expression, all forms warrant systemic evaluation for syndromes or associations.

- Ulnar deficiency

Ulnar deficiency is 4−10 times less common than radial deficiency. This anomaly affects the postaxial border of the limb. Unlike radial deficiencies, ulnar deficiencies are not associated with systemic conditions.

Central deficiencies

- Split hand−split foot

This is a longitudinal-mesoaxial defect of the distal limbs that has clinical expressions that range from absence of the central rays to more severe forms, such as ulnar monodactyly. It can occur as an isolated defect, restricted to distal limbs, or as part of syndromes, such as the ectrodactyly-ectodermal dysplasia-cleft palate syndrome, limb-mammary syndromes, and other conditions

- Syndactyly

Syndactyly is defined as an abnormal interconnection between adjacent digits and is described according to the magnitude and extent of the linkage. The interconnection may encompass the entire length of the adjacent digits (complete) or it may discontinue proximal to the fingertip (incomplete). The syndactyly may involve only skin and fibrous tissue (simple) or include bone (complex). Syndactyly that occurs with other anomalies (e.g. Apert syndrome) is referred to as complicated syndactyly. Syndactyly is a common congenital anomaly, with an incidence of approximately 2−3 per 10 000 live births.

- Polydactyly

Polydactyly can occur on the preaxial (radial) and the postaxial (ulnar) side of the limb. Central polydactyly is an extra digit within the hand and not along its borders.

The central polydactyly may be hidden within a concomitant syndactyly (i.e. synpolydactyly).

Dr Latos-Bielenska reported that there are 123 registries of limb malformations. The International Clearinghouse for Birth Defects Surveillance and Research has 47 members worldwide, while EUROCAT has 43 member registries in 20 countries. She also drew attention to the differing legal situation regarding termination of pregnancy for fetal anomalies in different countries. Cases of limb reduction defects were shown to vary from around 2 to 12 per 10 000 births in 22 European countries. A hospital-based malformation surveillance programme in Boston, USA, during 1972−1974 and 1979−1994 showed that the prevalence rate for all types of limb deficiency was 6.9 per 10 000. She noted that in Poland children with limb reduction defects often have other congenital defects.

In conclusion, Dr Latos-Bielenska suggested that It would be valuable to develop a uniform system of surveillance of limb reduction defects (with a detailed description of each limb defect using schematic diagrams, appropriate coding, a detailed interview about maternal medication during pregnancy, information on consanguinity and family history, a clinical examination by a geneticist, and appropriate genetic tests). She also noted that it would be helpful to introduce biobanking of DNA samples in order to speed up studies on the molecular basis of limb reduction defects.

In discussion, it was pointed out that it would be useful from the WHO perspective if the meeting could consider issues beyond thalidomide. It was also felt that it would be helpful to have information on different populations around the world. It was noted that Poland has collected data on limb reduction defects over a number of years but that there seems to be little change in prevalence over time.

Mechanisms of the effect of thalidomide

This session involved one presentation on limb development followed by three presentations on the different influences of thalidomide.

Limb development

Dr Malcolm Logan of Kings College London, United Kingdom, described the development of the human embryo at various time points, showing the early stages of limb development. It is at these early stages that it is thought thalidomide has its effect. Animal models used in early-stage embryo research are frogs, chickens, mice and even fish whose fins have some basic similarities to human limbs. Meeting participants were shown a series of images of mouse embryos illustrating the very early stages of development of the limbs.

It was explained that there is an initiation event which starts the recruitment of cells to form the limb buds, and then these develop with patterning and morphogenesis into distinct limb elements. The secretion of signalling molecules called fibroblast growth factors (FGFs) is able to induce ectopic limb formation from cells in the flank of the chick embryo. Indeed, one FGF molecule is enough to induce a series of events that lead to limb formation. Cells in the flank respond to this secreted signal, but it has been shown that if FGF 10, which is expressed in the pre-limb bud mesenchyme, is prevented from influencing the flank cells, the fetus develops without limbs. If there a positive feedback loop of FGF signalling (FGF 10 and FGF 8) between the mesenchyme and ectoderm, however, that will drive the growth of the limb bud.

Dr Logan also pointed to the importance of genes *tbx 4* and *tbx 5* which are expressed in the hind limbs and fore limbs respectively. Deleting these in animal models led to the absence of hind limbs and fore limbs. For instance, the deletion of *tbx 5* in animal models led to disruption of FGF signalling and a failure of initiation of the fore limbs. He further noted that some secreted proteins called Wnts also have an effect by inducing the formation of ectopic limbs from the inter-limb lateral plate mesoderm. Further, disruption of retinoic acid production through deletion of the biosynthetic enzyme Raldh2 also causes limb abnormalities. Thus, it was noted, multiple signalling inputs converge to enable limb bud initiation to begin, and the absence of any of these can inhibit limb development.

Once the limb bud exists, three signalling centres interact but if one centre is affected there is a knock-on effect on the others. Outgrowth of the limb bud develops from the upper arm to the forearm and to the hands. Phocomelia can arise following disruption of (early) events in limb mesenchyme which may be due to a combination of failure to recruit sufficient precursor cells and disruption of correct proximal-distal patterning. Disruption of proximal-distal signalling will have consequences for activator protein signalling. Disruption of FGF signalling from the apical ectodermal ridge produces predominantly distal defects but not classic phocomelia.

In discussion it was noted that the loss of proximal and distal limb structure could be due to a variety of mechanisms. It was also pointed out that, with the disruption of FGF signalling, the left limb is always more affected than the right; however, this does not seem to be seen in thalidomide embryopathy. Differences were stressed between rat and mouse embryos during development, with asymmetrical development in the mouse in the early stages.

Angiogenesis

Dr Neil Vargesson of the University of Aberdeen, United Kingdom, addressed the issue of angiogenesis – the process by which new blood vessels form from existing vessels.

While vasculogenesis is the formation of the first embryonic vessels by recruitment of endothelial cells, angiogenesis is the process by which these initial blood vessels are then elaborated and new ones can form to vascularise growing tissues. It is an essential process for normal growth. However, cancers also grow through angiogenesis. Experiments with chicken and zebrafish embryos, rodents, humans suffering from vascular disorders and in vitro cell cultures have shown that thalidomide is anti-angiogenic, and for that reason the drug is nowadays used in treating cancer.

Thalidomide breaks down in different ways in different parts of body, and is known to have at least 20 metabolites and analogues. The anti-angiogenic thalidomide analogue CPS49 is one that causes problems in early-stage limb development. Experiments in which CPS49 was applied in the early stages of limb formation resulted in a range of limb anomalies within a one-day period in chicken and zebrafish embryos.

Meeting participants were shown the results of experiments using thalidomide and thalidomide analogues on chicken, marmoset and monkey embryos. The effects of CPS49 were typically very rapid, resulting in limb damage very similar to that seen using thalidomide and other thalidomide analogues in chickens, zebrafish, monkeys and marmosets. It was also pointed out that thalidomide causes eye, ear facial and internal organ problems as well as reduced limbs. However, Dr Vargesson stressed that the non-anti-angiogenic analogues of thalidomide do not cause deformities.

Dr Vargesson reported that the development of smooth muscle seems to protect against thalidomide. Early limb vessels lack smooth muscle although it is present on most other vessels of the embryo. The action of CPS49 depends on the stage of development at which it is introduced; the earlier the stage of development, the more destructive the impact of CPS49 due to the lack of smooth muscle on vessels earlier in development. In zebrafish embryos, CPS49 leads to prevention of the development of filopodia, which are extensions from the endothelial cells that migrate to form connections and new vessels with other endothelial cells. CPS49 prevents new vessel formation. Experiments have shown that CPS49 not only reduces blood vessels but also causes cell death and reduces or causes the loss of key signalling molecules involved in limb development, including Shh, FGF 8 and FGF 10. However the induction of cell death and loss of signalling molecules occurs after the effects on blood vessels. A similar effect has also been observed by thalidomide in chicken, zebrafish and rabbit embryos.

A number of potential targets of the action of thalidomide have been proposed, including cereblon, nitric oxide, reactive oxygen species, the cytoskeleton and FGFs. However, researchers have highlighted over 2000 gene profiles (many of them cytoskeletal or vascular) that were changed in tissue treated with thalidomide.

In summary Dr Vargesson explained that, when thalidomide is used in animal experiments, anti-angiogenic could inhibit the formation of new blood vessels and in turn cause cell death. The loss of cells then results in defects in the development of

organs or other tissues, which in turn leads to defects in secondary cell induction (such as innervation).

Cereblon

Dr Hiroshi Handa of Tokyo Medical University, Japan, introduced the issue of cereblon as a target of thalidomide teratogenicity, in particular by the use of affinity bead technology. He outlined the development of two types of submicron beads (latex SG beads and magnetic FG beads) with similar surface properties for affinity chromatography, describing their high recovery, high purity and general versatility.

Dr Handa explained that single-process affinity purification ensures high recovery and quick identification of drug-binding proteins, evaluation of binding specificity by competitive experiments, and determination of binding modes and affinity. Using this method, the Japanese researchers studied a number of ligands, including thalidomide. In particular the research focused on identifying cereblon and the damage-specific DNA-binding protein 1 (DDB1) as thalidomide-binding proteins.

Cereblon forms a stable complex with DDB1, which is a component of the E3 ubiquitin ligase complex. However, cereblon directly binds to thalidomide, which in turn affects the enzymatic activity of the ubiquitin ligase complex. Investigations were carried out to ascertain a possible connection between cereblon and thalidomide teratogenicity in zebrafish, with developmental defects to the pectoral fin and otic vessel indicating that the cereblon-containing E3 ubiquitin ligase complex, which is involved in early development, is also a target of thalidomide.

To try to confirm these results, the researchers identified a cereblon mutant that did not bind to thalidomide but was otherwise fully functional. Known as $CRBN^{YW/AA}$, the cereblon mutant was shown to suppress thalidomide-induced teratogenicity not only in zebrafish but also in chicken embryos. The Japanese research indicated that thalidomide's teratogenic effect occurs prior to angiogenesis, in which cereblon plays a role.

Cereblon contributes to normal development of limbs, at least in part by controlling the expression of FGF 8 from the apical ectodermal ridge. Thalidomide exerts teratogenic effects by binding to cereblon and altering the ubiquitin ligase activity. However, the research showed that $CRBN^{YW/AA}$ restored the expression of FGF 8 from the apical ectodermal ridge in chicken embryos.

In conclusion, Dr Handa discussed the use of immunomodulatory drugs (IMiDs), which are structural and functional analogues of thalidomide, including lenalidomide and pomalidomide. These have become important alternatives to thalidomide because of their more potent anti-cancer and immunomodulatory properties, as well as their improved side-effect profiles. He suggested that lenalidomide and pomalidomide are far

more effective than thalidomide in the treatment of multiple myeloma and myelodysplastic syndrome. Cereblon is a common target for IMiDs and is responsible for their antitumor effects on multiple myeloma, though different substrates for ubiquitination and proteasomal degradation are likely to be involved in the different pharmacological activities of IMiDs.

Neural crest

Dr Janet McCredie of the Faculty of Medicine of the University of Sydney, Australia, described the impact of thalidomide on neural crest, indicating that the effect of thalidomide precedes the development of the limb bud. Neural crest is a primordial element in the early embryo that leads to the development of a ganglion for the spine and for the bones of the face, as well as for other elements of the body.

Referring to a gestation timeline, Dr McCredie said that thalidomide has its teratogenic impact during an identifiable sensitive period. A gestation timeline showed that the effects of thalidomide were first recorded on day 24 and that the limb bud begins to develop on day 28. The neural crest develops on day 18. Thus it was suggested that the sensitive period for the effect of thalidomide was from day 21 to day 42.

Some 80% of human embryos develop the right and left limbs synchronously but 20% develop asynchronously with up to four days difference. Therefore, one could assume that 80% of thalidomide effects should be synchronous and 20% asynchronous but this does not seem to be the case.

An interesting factor is that, according to present knowledge, the central nervous system seems to be spared the effects of thalidomide. Meeting participants were shown maps of the arms and legs by sclerotomes which will not develop without sensory nerves. Research has shown that 80% of cases of limb defects match the lack of sensory nerve supply.

The physical impact of thalidomide can be seen in terms of sclerotome development. Absence of all or part of a single sclerotome reduces the mass and shortens the shaft of an affected long bone, resulting in hypoplasia or partial aplasia. Subtraction of more than one sclerotome causes further loss of bone mass, progressive shortening of long bones and increasingly severe reduction of the limb. A reduction in mass of the major long bones brings the hand or foot closer to the trunk (phocomelia). Neurotrosis fails in injured nerves, and bone fusion was seen in the effects of thalidomide. Over 90% of British thalidomide children with defective limbs also had internal defects.

Dr McCredie pointed out that the first negative effect of thalidomide to be noted was nerve damage in adults who had taken the drug for sedation. Neuropathy showed in patients only decades later.

Experiments on rabbits showed that a thalidomide-exposed litter would produce some with thalidomide syndrome deformities and some without. However, even the normal ones had neurological damage, although the deformed ones had most, and the damage appeared to predate the damage to limbs. In conclusion Dr McCredie proposed that thalidomide does not have an effect on blood vessels or genes, but that it damages nerves.

Factors to consider regarding the proposed mechanisms

Dr Nigel Brown of St George's, University of London, United Kingdom, summarized the factors to consider regarding the plausibility of the various proposed mechanisms of thalidomide embryopathy. He noted that it is clear that thalidomide has an effect on the embryo and that this effect may be on several elements, either individually or in combination with others. However, while it is certain that thalidomide has all the effects described by the earlier presenters, no single effect has been convincingly shown to be the cause of the thalidomide syndrome.

It was pointed out that thalidomide embryopathy can phenocopy genetic defects, thus highlighting the challenge in distinguishing thalidomide embryopathy from other problems. In view of the range of damage and variability between patients, it is therefore difficult to specify exact diagnostic parameters of thalidomide embryopathy.

Discussion included the fact that the effects of thalidomide are by no means limited to the limbs, and that the drug causes defects to the eyes, ears, central nervous system and face. There was general agreement that it is important to continue to bear in mind thalidomide's effect on organ systems. The question was also asked as to whether it is necessary to look for a common cause of embryonic damage, especially since the evidence shows that there appear to be many possible routes.

There were further questions as to whether researchers had seen a dose–response effect in any of their animal experiments. Additionally it was noted that the vascular structure of a thalidomide limb is very different from a normal limb and that this might be a possible area for research. It was also stressed that one major element in considering the impact of thalidomide is the host, and that addressing the host means dealing with probabilities.

Oxidative damage could come from anti-angiogenic effects, or it could result from another independent effect, and it must be borne in mind that there may well be a

number of different routes for the teratogenicity of thalidomide. Even the metabolites of thalidomide behave differently.

Participants felt that there are various tests that can be done to ascertain whether thalidomide is the cause of a physical defect. However, the major consideration must be exposure to the drug. It was pointed out that Nowack in 1965 included in his study only children born of mothers for whom exposure to thalidomide was confirmed.

Clinical observations

A historical review of thalidomide

Dr Emma Baple of St George's, University of London, United Kingdom, provided a historical review of the use of, and damage caused by, thalidomide. The drug was originally sold in Germany in the 1950s as a nonlethal sedative with no serious side-effects. However, the safety tests that were carried out were few compared to what would be expected today. Thalidomide was marketed by Chemie Grünenthal as a safe drug and was sold over the counter as Contergan in Germany. In the United Kingdom the Distillers Company marketed it as Distavel and promoted it for its safety.

Few side-effects of thalidomide were noticed until 1960 when it was found to cause peripheral neuropathy. The teratogenic effects came to be known in 1961 after women were treated with the drug for morning sickness. Lenz investigated an outbreak of limb defects in Germany. Germany had one reported case between 1930 and 1955 but eight cases out of 6000 births in a 12-month period in 1960–1961. Lenz reported his concerns to Chemie Grünenthal. Then, as a result of further cases coming to light, such as in the United Kingdom, and reports of concerns to the drug company, including from Australia where they were highlighted by McBride, the drug was withdrawn in November 1961.

Dr Frances Kelsey at the United States Food and Drug Administration (FDA) refused to license thalidomide in the USA. However, some clinical trials had been carried out there and 20 000 people, including an estimated 207 pregnant women, were given thalidomide.

Lenz went on to study cases, finding that in half of cases only the upper limbs were affected. He and colleagues did further investigations and suspected that the timing of the dose was critical and more important than the dose itself. This work resulted in a thalidomide embryopathy exposure timeline that is still accepted today. In the United Kingdom in 1964 the Ministry of Health published a report on deformities caused by thalidomide. Meeting participants heard sound recordings and were shown video clips relating to the discovery of the damage caused by thalidomide, the stigmatization of

patients (and especially their mothers), the ways that children learned to cope (and how some developed exceptional skills because of their disabilities).

Dr Baple pointed out that 50 years ago investigations were hampered by the fact that there was often lack of clear evidence of exposure to the drug, thalidomide was included in some medications but was not listed as present, birth defect records were often non-existent or incomplete, severe defects were recognized because they had previously been rare (but other defects were not necessarily noticed), and mothers' memories about when they used the drug and how much they took were frequently unreliable. In addition, molecular genetic testing was not available to help exclude phenocopies of thalidomide embryopathy such as Okihiro syndrome and Holt-Oram syndrome.

In 1994, following reports of affected offspring of possible thalidomide victims, McBride suggested that thalidomide might be a mutagen, but that theory was later disproved.

Dr Baple showed a series of examples of the differential diagnoses of thalidomide embryopathy and various genetic defects associated with limb defects, illustrating the large degree of overlap and the consequent difficulty in attributing a specific case to thalidomide. It was noted that in 1964 the United Kingdom government's report stated that "… it is impossible to say with any certainty what constitutes a thalidomide deformity ..."

Current experience of thalidomide effects

Dr Lavinia Schuler-Faccini of the Teratogen Information Service of Brazil's National Institute of Population Medical Genetics, based in Porto Alegre, reported on current experience of thalidomide-affected individuals in Brazil. She pointed out that this large country has around 190 million people and some 3 million births per year. There are large social and health differences.

During the 1960s about 1000 people in Brazil were affected by thalidomide and the drug was withdrawn in mid-1962. Then in 1965 some benefit was found in using thalidomide in leprosy patients. Brazil has around 35 000 new cases of leprosy each year, mainly in the north of the country. Thus thalidomide was never completely withdrawn as a drug in Brazil, but its use was shifted to leprosy. In 1996 it was reported that some 34 thalidomide embropathy cases had been recorded in South America since 1965 in areas where leprosy was present.

In Brazil today, thalidomide is approved for treatment of leprosy (more specifically, erythema nodosum leprosum [ENL] reactions), lupus, multiple myeloma, AIDS and a number of individual indications. The drug is not marketed but is produced by one laboratory solely for the government health service. It is given only by doctors of the

government health service and informed consent is required. Women of reproductive age are not included unless there are special circumstances.

Dr Schuler-Faccini gave examples of several recent cases of thalidomide syndrome in babies in Brazil. Among these were:
- a baby with upper and lower limb reductions, whose father was on thalidomide for ENL and whose mother took some of the tablets when she was sick and before recognizing she was pregnant (interruption of pregnancy is not allowed in Brazil);
- a baby born with upper limb defects and cardiovascular problems whose mother was being treated for leprosy and believed thalidomide had contraceptive properties (the baby died of its heart defect);
- a twin birth, of which the first twin was stillborn with no thumbs and kidney and heart damage, and the live twin had bilateral limb defects, whose young mother had psychiatric problems and took thalidomide (which had been given to her grandmother for myeloma) to try to abort the pregnancy;
- a baby with upper and lower limb deformities and microphthalmia of one eye, whose mother had leprosy and was not given thalidomide as she refused to use contraceptives, but whose father had obtained the drug for his wife unofficially;
- a baby with limb deformities, transverse limb defects and not typical of thalidomide embryopathy, whose mother had tried to terminate her pregnancy with a high dose of misoprostol.

Dr Schuler-Faccini and colleagues decided to screen babies born with limb reduction defects between 2000 and 2008 and found 3.1 cases of thalidomide embryopathy phenotype per 10 000 births.

Dr Eduardo Castilla of Brazil drew attention to the poverty and lack of literacy in very remote parts of the country. Leprosy is still endemic in many areas and thalidomide is prescribed for ENL. However, as in similar situations elsewhere, there is a tendency to share medicines with other members of the family. He noted that if it were not for the use of thalidomide for leprosy, there would be no cases of thalidomide embryopathy in Brazil.

In discussion it was pointed out that Brazil has no formal registry of congenital abnormalities. Consequently only certain cases are reported, with some cases coming to light when people hear about the possibility of compensation from the government.

Introduction to diagnosis

Decision trees

Dr Johan Ellenius of UMC explained that a decision tree is one example of a classification method. It is a decision-support tool that maps a case, characterized by a set of attributes to one of several predefined categories. The challenge is to find a model that does this effectively.

A decision accomplishes this by asking a series of questions. Starting with the first question (at the root node) each answer leads to further questions (at internal nodes) until the information is available on which to take a decision (at leaf nodes). Each internal node represents a test on an attribute while each branch from that node represents the outcome of the test. Each leaf node (where there are no more branches) represents a "class label" (i.e. a category of classification, suggesting a decision taken after considering all attributes).

A series of yes/no answers at a series of internal nodes based on a set of tests is the simplest form of decision tree. However, decision trees can also be used with bigger sets of data so long as each element can be measured. A decision tree can be either manually constructed or automatically built by applying data-mining algorithms. Such algorithms utilize a "learning" dataset of preclassified cases and produce a decision tree that can accurately reproduce the classification of individual cases. A challenge with this approach is that the resulting decision tree may be too complex because it is too closely adapted to the peculiarities of the cases in the learning set. A method to reduce the complexity of a decision tree and thereby improve its ability to classify new cases is to "prune" branches

Dr Ellenius showed the example of a decision tree developed to identify men with high risk of prostate cancer. The example was based on a study of 1433 men with elevated prostate-specific antigen (PSA) who underwent prostate biopsy. The factors analysed in the decision tree were age, PSA levels, percentage of free PSA in the blood, prostate volume, PSA density, and transrectal ultrasound. The decision tree was built on the basis of different tests for different groups in order to identify high-risk groups.

A diagnostic decision tree suggests a diagnosis. When evaluating the measures of diagnostic performance, diagnostic sensitivity and specificity characterize the performance on populations with and without the disease being tested for, and the predictive value is important for understanding to what degree a positive or negative test result can be trusted as correct in the clinical situation.

Benefits of a decision tree when compared with other, so-called "black box" models include the fact that the reasoning for a patient's classification can be tracked and printed on paper to be seen. On the other hand, not all classification problems can be

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solved by a decision tree. In discussion it was noted that there are often limited data to work with and that expert opinion may be resorted to if there is no gold standard for the decision. It was noted, however, that a decision-making process, such as that of a decision tree, may support the clinician in making a diagnosis.

Diagnostic algorithm for thalidomide embryopathy

Dr Sahar Mansour and Dr Christine Hall introduced the proposed diagnostic algorithm for thalidomide embryopathy (DATE), which is a computer software. St George's, University of London, had been requested by the Thalidomide Trust to prepare an independent review of thalidomide embryopathy (Annex 3) and in doing so had concluded that the only way to determine the minimum diagnostic criteria of thalidomide embryopathy would be through an international consensus between experts in the field. Accurate characterization of the "core" features of thalidomide embryopathy would lead to a better understanding of the underlying mechanisms of thalidomide embryopathy and targeted help and support for the victims of this condition.

In order to prepare the algorithm the St George's team had reviewed the existing literature with respect to the clinical and radiographic features of thalidomide syndrome, reviewed a random selection of beneficiaries from the Thalidomide Trust and their radiographs, consulted *Aids to diagnosis* (by Dr Claus Newman), and reviewed the frequency of each phenotypic feature in other syndromes and in the general population.

All the features related to thalidomide embryopathy were reviewed and each feature was attributed a weighted score according to whether it occurred rarely without thalidomide (high score) or was common even without thalidomide (low score). It was acknowledged that, as far as concerns the review of the literature and beneficiaries, much of the required information was not available, there were no full skeletal surveys, some cases were included which clearly had alternative diagnoses, and it was rare to have actual evidence of thalidomide ingestion during pregnancy (it was often presumed to have taken place).

The computer program was demonstrated. The first page asked for data on family history of defects, maternal thalidomide ingestion (high score), maternal medical condition or exposure to another teratogen, and genetic tests performed. This basic information was followed by a series of 64 questions. Meeting participants were shown the different stages of the algorithm and were shown sample questions, many of them along the lines of "does the patient have …?" or "is there evidence of ...?" or "is there any ...?", with higher scores allocated for each answer considered to be predictive of thalidomide embryopathy. Definitions of the conditions being asked about were included in the algorithm. It was pointed out that the scoring had yet to be finalized. Once final, the program should be able to offer a list of differential diagnoses for other syndromes.

Workshops on diagnostic criteria and on thalidomide mechanisms

On the second day of the meeting, participants spent much of the time in one of two workshops – on diagnostic criteria and on thalidomide mechanisms.

Diagnostic criteria workshop

In order to test the new algorithm (DATE) developed by St George's, University of London, clinicians from several countries presented clinical details and radiographs of cases. The information presented was entered into the algorithm in order to provide feedback on its effectiveness and on the appropriateness of the questions and scoring. The aim was to assess the effectiveness of the algorithm in processing data from different countries and to find out whether any elements should be added, removed or scored differently. The algorithm's scoring system attributed scores of varying weights to the different responses in an attempt to classify cases as due to thalidomide or not. The computer program also included differential diagnoses for other syndromes.

The chair reminded participants that the aim of the workshop was to define the core features of thalidomide embryopathy (i.e. those features that are highly likely to be caused by thalidomide), to identify the features that are highly unlikely to be caused by thalidomide, and to identify grey areas. The cases submitted were anonymous and the discussions confidential. The sole purpose for the cases being described was to assess the validity and usefulness of the proposed diagnostic algorithm (DATE).

Clinicians presented two cases from Australia: a 35-year-old man born in 1979 with thalidomide-type deformities, and a 20-year-old woman who had a pregnancy terminated due to the fetus developing without limbs.

Five cases were presented from Germany: a baby born in mid-1962 with limb defects and whose mother was known to have taken thalidomide; a woman born in January 1962 with foot and hand defects and deafness but no evidence that her mother used thalidomide; an adopted child born in Turkey in 1972 with a range of limb defects and raised in Germany; an Italian female with deformities to the legs and uterus born in 1963 (thalidomide was available in Italy up to 1962) whose mother died when the girl was 7 years of age and with no evidence that the mother took thalidomide; and a German female with minor problems of the upper limbs who was born in September 1961 and whose mother took one and a half thalidomide pills, probably in the first trimester.

Four cases were presented from Brazil: a baby born in 2010 with severe restrictions of the upper limbs and deformities of the lower ones whose mother took thalidomide without realizing she was pregnant; a baby born in 2005 with very short legs and deformities of the hands but with no evidence that the mother took thalidomide; a baby with considerable damage to the arms (but none to the legs) and with inguinal hernia

and absence of gonads but with no photographs or other information available; and a child born with a defect to one arm but no connection to thalidomide.

Several further cases were presented from Sweden: a man with no or limited thumbs and limited movement of one eye, whose mother took two thalidomide tablets; a woman aged 27 who died a year ago and who had limited eye movement, ear malformations and deafness; and a 28-year-old autistic man with severe ear damage, no movement in one eye, facial palsy, deafness, tearing when eating, and deformity of both thumbs and the left upper limb. Given these features and the exposure timeline, it was suggested that the mother took thalidomide in the first month of pregnancy. However, there was no documented evidence of this and, in view of the significant dysmorphic facial features of the man, delegates felt that an alternative diagnosis should be considered.

These cases were each discussed in detail in order to enter details into the algorithm as fully as possible. The cases gave rise to proposals for adjustment of the scoring of certain elements, for expanding or refining some of the questions, for some changes to terminology, and for a set of exclusion criteria. There was also discussion of what to do with cases that fall into the area of uncertainty. Having reviewed the cases it was felt that some details may have been missed in some of the historical cases since, if there are limb deformities, patients may not be examined for other problems.

The team from St George's, University of London, recorded all proposed changes to the algorithm and agreed to update the computer program in accordance with the feedback received. The meeting felt that, once revised in line with participants' suggestions, the algorithm could be used to assess a larger number of cases in order to propose diagnostic criteria for thalidomide embryopathy. Cases would be classified as highly likely, probable, possible, unlikely, and inconclusive where the data are insufficient. The aim would be to select a cohort of "highly likely" cases with as much evidence of exposure and clinical data as possible and with radiographs in order to identify the core features of thalidomide embryopathy. It was hoped that sufficient data existed to put together 200 reliable cases for this exercise. Dr Claus Newman pointed out that a set of such cases existed, as exemplified in the 1965 paper by Nowack[1] and additionally by those of Lenz & Knapp[2] and by Kreipe.[3] Dr Newman was requested to translate Nowack's 1965 paper into English (the papers by Lenz & Knapp and by Kreipe having already been translated) as it contained high-quality case reports that would be important in the development of the algorithm.

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[1] Nowack E. Die sensible Phase bei der Thalidomid-Embryopathie. Humangenetik, 1965, 1:516–536.
[2] Lenz W, Knapp K. Die Thalidomid-Embryopathie. Deutsche medizinische Wochenschrift, 1962, 87(24):1232–1242.
[3] Kreipe U. Misbildungen innerer Organe bei Thalidomidembryopathie. Archiv für Kinderheilkunde, 1967, 176:33–61.

Once a clear standard for the diagnosis of "highly likely to have thalidomide embryopathy" is agreed, epidemiological and other issues can be taken into consideration to define the score for the "probable", "possible" and "highly unlikely" categories. It was noted that some funding might be available from UMC to take the work on a decision tree forward.

Thalidomide mechanisms workshop

Dr Paris Ataliotis of St George's, University of London, reported to the plenary on the discussions of the workshop on the mechanisms of action of thalidomide. He emphasized that there had been very fruitful discussions and that a number of those present had shared unpublished data and had already agreed to be in contact in future to facilitate each other's work. Additionally they had identified several areas for further research.

Stressing the importance of understanding the mechanism by which thalidomide causes limb reduction and other deformities, Dr Ataliotis reminded meeting participants of the process of initiation of the limb bud in the embryo and of the genes required for the initiation to take place.

Cereblon is involved in the degradation of protein substrates in the cell cytoplasm by adding the regulatory protein ubiquitin to them (a process called ubiquitination). In the presence of cereblon, FGF signalling occurs, and if cereblon is removed FGF signalling stops. If thalidomide is added to an embryo, the activity of cereblon is blocked so that the normal degradation of cell substrates no longer takes place. However, there is a possibility that the specificity of action may have changed, so that things that are not normally degraded by cereblon now become degraded, and perhaps even that things normally degraded by cereblon are now degraded at different rates.

Adding thalidomide to the embryo not only blocks the activity of cereblon, but it also stops FGF signalling and limits angiogenesis, though it is unclear in which order these occur. Other targets of thalidomide have been identified and they also involve ubiquitination which alters their rates of degradation. One of these targets has been shown to be upstream of angiogenesis. Thus the group discussing mechanisms concluded that thalidomide's action on cereblon and angiogenesis seems to be significant and is an area that calls for further investigation.

Dr Handa had already showed that cereblon binds to thalidomide directly but if human cereblon is mutated by certain amino acids the action of binding to thalidomide can be blocked. However, mutations are found in human cereblon that do not cause limb defects. Mice and rats also have cereblon that binds to thalidomide, but there is a specific amino acid outside the thalidomide binding domain which, it has been suggested, may change the specificity of the thalidomide substrates.

The group considered whether there may be tissue-specific cereblon substrates. Cereblon is widely expressed in the human body yet the limbs are primarily affected by thalidomide, but it is unclear why. Perhaps, the group felt, the cereblon substrates may differ from one tissue to another.

Several areas for research were outlined that the participants in the thalidomide mechanisms workshop considered need to be done and that the group felt capable of doing. The research would focus on three areas, namely:
- **The tissue-specificity of cereblon substrates.**
- **The human cereblon mutation (including the possible presence of protein from patient cells, the impact on fish defects, and whether it can rescue cereblon knockdown cells).**
- **Species sensitivity and its link to rodent polymorphism (including humanized cereblon in the mouse).**

By getting a clearer idea of the mechanism of action by thalidomide, one would have a clearer idea of the diagnosis, Dr Ataliotis concluded.

Closing

The chair thanked all participants for their contributions to the plenary sessions and the two focused workshops. He thanked the Thalidomide Trust for raising concern about the lack of precise diagnostic criteria for thalidomide embryopathy, and he thanked St George's, University of London, for doing a large amount of work in a very short time and for guiding the meeting towards a future diagnosis. It was noted that UMC would keep the meeting SharePoint site open with alerts to meeting participants when new information was added.

On behalf of WHO, Dr Shanthi Pal thanked participants for the collegial atmosphere in which they had conducted the meeting and their passion for taking the study of the issue further. She also thanked the WHO support team. Dr Pal stressed that the meeting should not be considered the end of WHO's involvement but as the first step in this collaborative process. As the work progressed there would be consideration of how to give it more visibility in WHO fora.

Summary findings and recommendations from the meeting

By Ralph Edwards and Sten Olsson

Below are some consensus points distilled, mainly from the sections in **Bold** text in this report:

- The diagnostic criteria used currently and collected into the St George's algorithm (DATE) resulted in:
    - High scores in cases with clear exposure to thalidomide
    - Much lower scores in uncertain cases
    - Differentiation that allows the algorithm to be used to aid diagnostic decisions.
- The algorithm is a strong basis for a decision tree that can developed and validated using some 200 old and contemporary cases with near certainty of maternal thalidomide intake, at a relevant time in pregnancy
    - Agreed work to be done includes
        - Assembly, translation and analysis of cases by the St. Georges team
        - Development and testing of a decision tree by the UMC team
    - Funding should be provided urgently.
- The current experimental work presented at the meeting does not influence the human clinical diagnostic criteria of thalidomide embryopathy.
- The experimental work suggested by the mechanisms workshop should be supported because of the profound nature of thalidomide effects on the understanding of fetal development, genomics and proteomics, leading to potential beneficial, safe use of thalidomide and its analogues in cancer treatment and in autoimmune disease
    - The small group of mechanisms experts will work together to forward this aim.
- Increasing availability of thalidomide, particularly its use in countries with limited drug regulation (because of its important use in leprosy treatment), is the cause of a new wave of fetal malformations, either during a clinically indicated use or by accidental/off-label use by unintended users
    - It is important that all concerned with the promotion, prescription and dispensing of thalidomide take utmost efforts and care in warning of the dire consequences of its use in pregnancy
    - Manufacturers of thalidomide must make clear the grave risks of even tiny doses on the fetus
    - Every effort should be made to warn against and prevent the diversion of thalidomide to unwary third parties with trivial, off-label indications for its use.
- An effort should be made to gather complete, contemporary, global information on the incidence and prevalence of thalidomide fetotoxicity

- The most complete possible drug exposure histories for the whole of pregnancy and the weeks prior to conception must be recorded
- Genetic testing and clinical genetic evaluations should be done wherever possible
  - There should be meticulous recording of all clinical and special investigation findings.

Annex 1. Participants in the Thalidomide Embryopathy Meeting of Experts

Geneva , 2014

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