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Assessment report Immunological differences of pandemic vaccines (review of hypothesis on Pandemrix and development of narcolepsy)

Review under Article 5(3) of Regulation (EC) No 726/2004

Procedure number: EMEA/H/A-5(3)/1343

Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. Background information on the procedure

The European Medicines Agency (EMA) was made aware of preliminary laboratory results comparing the immunological properties of Pandemrix and Arepanrix[1], (H1N1)v AS03[2] adjuvanted vaccines. The report received addressed initial findings based on *in vitro* studies carried out by the National Institute for Health and Welfare (THL) in Finland using plasma samples from patients who developed narcolepsy and healthy vaccinees following vaccination with Pandemrix. The Agency was also made aware of updated recommendations on seasonal influenza vaccination within the Finnish national vaccination program for the season 2012/2013 issued by THL[3] on 10 October 2012.

A study in Finland has been conducted in children with narcolepsy, diagnosed after Pandemrix vaccination who were recruited in Finnish children's hospitals during 2011. All children were vaccinated by the end of year 2009 and the blood samples were collected during year 2011. The authors suggest that their data show that children who developed narcolepsy after Pandemrix vaccination developed an altered immune response, and that polysorbate 80 is an immunologically active substance in the H1N1 antigen suspension of Pandemrix. They hypothesise that polysorbate 80 in H1N1 antigen suspension of Pandemrix revealed new epitope(s) in the viral protein(s), and the antibodies against these epitopes cross-reacted with narcolepsy associated autoantigens. The authors therefore claim that the observed immunological differences between Pandemrix and Arepanrix antigen suspensions could explain the differences in the risk of narcolepsy between Pandemrix and Arepanrix.

The hypotheses seem to rely on two key assumptions, namely that the presence of polysorbate 80 in the Pandemrix antigen suspension could be the only 'immunological' difference between Arepanrix and that Arepanrix is not associated with a risk of narcolepsy.

The authors conclude that, according to their hypothesis, it cannot be excluded that other H1N1 vaccines with similar viral antigens (i.e. similar production method of viral antigen) could trigger narcolepsy especially in children primed earlier with Pandemrix vaccination.

In light of this new data, the Executive Director of the EMA presented on 15 October 2012 a request for a Committee for Medicinal Products for Human Use (CHMP) opinion under Article 5(3) of Regulation (EC) 726/2004. A CHMP opinion was sought on several questions as detailed hereinafter in this report. The assessment of the initial findings of the *in vitro* studies carried out by THL was considered for this procedure.

2. Scientific discussion

2.1. Introduction

On 15 October 2012 the Executive Director of the EMA asked the CHMP, in accordance with Article 5(3) of Regulation (EC) No 726/2004, to give an opinion on questions regarding immunological differences identified in pandemic vaccines, further to the availability of initial results of a study performed in Finland. This report considers the preliminary results of this study, and the answers to the questions put to the Committee should be read in light of the evidence available to date.

2.2. Discussion

2.2.1 Question 1
Are the methods and assays used valid, and sufficiently specific, to identify a specific antibody induced by Pandemrix?

The study performed by the investigators' team used an enzyme linked solid-phase immunoassay (EIA) for detecting binding of IgG antibodies to AS03 and its components. In brief, polystyrene plates were

[1] Pandemrix and Arepanrix are manufactured by the same marketing authorisation holder at different locations. The products contain the same adjuvant (AS03) but the antigen is produced using different manufacturing steps. Amongst other differences, the antigen suspension for Pandemrix contains polysorbate 80 as an excipient; Arepanrix's antigen suspension does not contain polysorbate 80.
[2] The AS03 adjuvant is composed of squalene, DL-α-tocopherol and polysorbate 80.
[3] The information published by THL can be found at http://www.thl.fi/en_US/web/en/news?id=31176 .

coated with ASO3 or other mixtures overnight. The plates were post-coated with fetal bovine serum (FBS). Plasma samples diluted 1:100 in FBS were incubated in the plates and IgG antibody binding detected using alkaline phosphate conjugated anti-IgG. The results were expressed as arbitrary optical density units.

In this assay the investigators focused on detecting a possible antibody response to the AS03 adjuvant and to determine if the response was different in children who developed narcolepsy after exposure to Pandemrix. The assay chosen relied on coating polystyrene plates with AS03 adjuvant and measuring the binding of antibodies in plasma to the components bound to the plate. A limitation of this assay format is that it measures binding of a complex mixture of antibodies to a mixture of antigens adsorbed onto the ELISA plate in undefined quantities. Based on the available data, the specificity of binding, or what components of the adjuvant the antibodies might be binding to cannot be ascertained.

The data are presented as preliminary and there remain a number of methodological issues with this assay.

Antigen binding

Similar assay formats are used routinely for measuring binding to protein antigens however, AS03 as an oil-in-water emulsion presents particular methodological issues. The performed experiments were done with very high non-standard coating concentrations of antigens. It is not known which components or how much of the AS03 mixture have bound the plates. The major components of AS03 are extremely hydrophobic and the potential for components of AS03 to bind non-specifically to other molecules with hydrophobic moieties such as some antibodies is very high.

Squalene and tocopherol are not soluble in water while polysorbate 80 is highly soluble in water. The development of a squalene ELISA has previously been described (*Matyas et al*., Journal of Immunological Methods 2004, 286:47-67). This report clearly shows that in order to obtain a specific squalene ELISA the coating process required the use of a solvent (isopropanol), special ELISA plates and the identification of an optimal blocking agent (*Matyas et al*., Journal of Immunological Methods 2004, 286:47-67). It is not clear if the investigator has taken this into account. By adding squalene and tocopherol as oils directly to PBS (water) without the presence of an emulsifier (polysorbate) the coating of plates will result in an unpredictable and unspecific assay.

Given the extreme hydrophobicity of the AS03 components there is a high probability of detecting non-specific binding to IgG. Low affinity antibodies would not have significant biological relevance. Such low affinity interactions are normally prevented by the use of low levels of surfactant such as polysorbate 80 in immunological assays. When developing novel immunological assays these issues are addressed by testing a range of blocking buffers which contain detergents, purified proteins or serum. In this assay the investigators have evaluated fetal bovine serum (FBS), bovine serum albumin (BSA) and casein.

Fetal bovine serum dependent binding of IgG to AS03

The binding of IgG to AS03 on solid phase was observed to be dependent on the presence of fetal bovine serum (FBS) in the post-coating and plasma dilution buffers in the AS03-EIA whereas, weaker or no IgG binding was seen when BSA or casein was used. The investigators interpret this finding as suggesting that serum derived protein is needed for the formation of the antigenic complex for antibodies in the assay, and that the antibodies do not recognise AS03 alone.

The requirement for a second coating layer to obtain binding is unusual and non-standard. FBS is not ideal as an immunological blocking buffer. The fetal bovine serum dependent binding of IgG to AS03 is an interesting observation but given the complex nature of FBS there could be a number of explanations for this finding. It could be interpreted at this stage that blocking buffers containing casein and bovine serum albumin are preventing non-specific binding in this assay and FBS is producing a false-positive.

There is no binding detected with serum to tocopherol nor squalene, with FBS saturation. However, as these oils are not soluble in water it is not clear if any of these components have bound the plates.

There is no increased binding (in narcoleptic children versus healthy control children) detected with polysorbate 80 + FBS saturation. This binding is observed in normal and disease samples despite the presence of soluble 1% FBS in the diluting buffer.

This data remains consistent with low affinity non-specific binding.

Reproducibility

The data is expressed as optical density (OD). Results in OD values for a single sample dilution cannot be interpreted in the absence of a reference curve, which was not available at this time. To allow proper statistical assessment of the data additional dilutions should be made and titers calculated.

Average background optical densities for plasma binding to uncoated plates are subtracted from the reported values. In this assay the background values appear high as the mean is reported by the investigators to be approximately OD 0.22. The apparent high background binding is not ideal when detecting changes in OD of a similar magnitude.

In this assay the higher binding AS03 “positive” serum is detected against a very high background of antibody binding to AS03 as evidenced by the high ODs in "negative" serum. In general there appears to be highly overlapping binding in both healthy children and children with narcolepsy.

It is not possible to conclude on the specificity of the assay based on the data available to date; additional experiments are required to demonstrate that the observed IgG binding in this assay is not the result of non-specific interactions.

In summary, based on the information available the assay cannot at this stage be considered sufficiently specific to identify a specific antibody induced by Pandemrix.

2.2.2 Question 2

Do the results provide robust evidence of a specific and differential immune response in narcolepsy patients and healthy vaccinated people, who have received Pandemrix?

A key finding of the Finnish study was that there were higher levels of IgG antibodies binding to AS03 in plasma from children with narcolepsy and exposed to Pandemrix compared to non-affected Pandemrix vaccinated children.

The titers were not normally distributed so the investigators log transformed the data. After this transformation the authors assumed that a parametric test was appropriate. It could be suggested that serological assays of this type should be analysed by use of non-parametric tests (e.g. Wilcoxon).

If the assay was considered specific and properly validated (data not available at this time) it could only be concluded that a sub-population of the narcoleptic children show increased levels of IgG binding to AS03, whereas the majority of the narcoleptic children do not show this increase, which questions whether this observed increase is effectively linked with narcolepsy.

The investigators show there is no significant difference in binding to AS03 between DQB1 *0602 positive healthy children exposed to Pandemrix and DQB1 *0602 positive children with narcolepsy exposed to Pandemrix.

Given the importance of HLA type in determining antibody responses the matched HLA group are the most relevant control and this observation raises questions over its relationship to narcolepsy.

In summary, based on the information available, the specificity of the antibodies identified remains unknown and there is no evidence of a specific and differential immune response in patients who developed narcolepsy and healthy vaccinated subjects, who have received Pandemrix.

2.2.3 Question 3

Do the results provide robust evidence of a different and specific immune response to Pandemrix versus Arepanrix?

The investigators wanted to assess if the AS03 binding antibodies they had identified would be inhibited by the Pandemrix and Arepanrix antigen suspensions (without adjuvant).

They found a very significant difference in the response to the two mixtures. They were able to show in this assay format that Pandemrix inhibited binding to AS03 whereas Arepanrix did not.

The inhibition of antibody binding to AS03 by Pandemrix antigen and not Arepanrix is key to the hypothesis developed by the researchers that there is an immunological difference between Pandemrix and Arepanrix and the role of polysorbate 80.

The investigators compared the Pandemrix and Arepanrix antigen suspensions for their ability to inhibit the binding of antibodies to AS03 from 6 children with narcolepsy displaying high serum binding to AS03. They found that a 1:10 dilution of Pandemrix antigen suspension inhibited the binding of antibodies to AS03, whereas Arepanrix antigen suspension did not.

The antigen component of Pandemrix differs from Arepanrix. In particular Pandemrix antigen suspension contains polysorbate 80 and Triton X-100 as excipients in similar amounts.

The link the investigators make to polysorbate 80 does not seem to take account of the potential of detergents such as polysorbate 80 to block or change hydrophobic interactions. As the researchers added these antigens in a 1:10 dilution there remains a relatively high concentration of detergent in the assay. In these circumstances it cannot be excluded that these surfactants are simply blocking low affinity non-specific antibody binding to “AS03” in this assay rather than blocking a specific immunological interaction as proposed by the investigators. There is no data available to date on the effect of polysorbate 80 or other detergents in this assay.

Low affinity interactions could equally also be inhibited by chaotropic ions or pH changes. This could also affect the inhibition experiments with complex vaccine formulations (including excipients) such as Arepanrix or Pandemrix.

In summary, the available data do not allow the conclusion that the assay is specific; it is also noted that the data are preliminary, and not yet validated; it cannot be considered that the available results provide robust evidence of a different and specific immune response to Pandemrix versus Arepanrix.

2.2.4 Question 4

Do the results provide robust evidence of a specific antibody to polysorbate 80-antigen complex?

There are concerns that the assay format is not specific or properly validated at this stage. There is no increased binding (in narcoleptic children versus healthy control children) detected with polysorbate 80 + FBS saturation. There is no data on the relative affinity of IgG binding to polysorbate + FBS. The hypotheses the investigators develop for a specific antibody to polysorbate 80-antigen complex relies largely on an assumption that the differential presence of polysorbate 80 in Pandemrix and Arepanrix is the only ‘immunological’ difference between the vaccines. As discussed in question 3, the available data do not lead to the conclusion that the identified antibody binding to “AS03” by Pandemrix and not Arepanrix is specific to a polysorbate 80/H1N1 complex.

In summary, based on the available data the specificity of the antibodies identified remains unknown. There is no evidence at this time of a specific antibody to a polysorbate 80-antigen complex.

2.2.5 Question 5

Has it been adequately established that polysorbate 80 is a key determinant in the differential immune responses for Pandemrix versus Arepanrix?

The hypotheses the investigators developed for a specific antibody to polysorbate 80-antigen complex relies largely on an assumption that the differential presence of polysorbate 80 in Pandemrix and Arepanrix is the only ‘immunological’ difference between the vaccines. As discussed in question 3, the available data do not lead to the conclusion that the identified antibody binding is specific to a polysorbate 80/H1N1 complex.

The hypothesis of a neo-epitope created on H1N1 in the presence of polysorbate 80 is not supported by the present data. Other studies, including structural analysis, would be needed to confirm this. The hypothesis would also have to incorporate how this epitope was revealed only in the antigen component and not when combined with the adjuvant, which contains much larger amounts of polysorbate 80.

In summary, the available data are insufficient to establish that polysorbate 80 is a key determinant in the differential immune responses for Pandemrix versus Arepanrix.

2.2.6 Question 6

Do the results provide any evidence to suggest that the antibody identified may be a factor in the development of narcolepsy?

It is acknowledged that the authors state that they do not consider the antibodies identified as pathogenic antibodies in narcolepsy, but suggest that these antibodies serve as a marker of altered reactivity to Pandemrix in these patients. The investigators appear to have studied the antibody response to the components of Pandemrix vaccination in order to use these antibodies as a surrogate marker for altered immunological reactivity to Pandemrix vaccination.

However, to answer this specific question, the available data do not provide evidence that the antibodies are either specific to polysorbate 80, or that they may be a factor in the development of narcolepsy. Regardless of the specificity of antibodies identified, a role in the development of narcolepsy cannot be evaluated.

The finding that some IgG antibodies can bind AS03 in Pandemrix-vaccinated cases might not be unexpected. However, there is no robust evidence that the antibodies identified in this study are specific for AS03. Antibodies to squalene have previously been detected in sera from both vaccinated and non-vaccinated subjects (*Matyas et al*., Journal of Immunological Methods 2004, 286:47-67 and *Del Giuduce et al*., Clin. Vaccine Immunol. 2006, 13(9):1010-1013) and thus it cannot be excluded that antibodies to squalene or AS03 are detected in subjects vaccinated with Pandemrix. A study by *Del Giuduce et al*. (Clin. Vaccine Immunol. 2006, 13(9):1010-1013) concluded that anti-squalene antibodies are not increased by immunisation with vaccines containing the MF59 adjuvant. However, the preliminary data does not allow conclusions on whether AS03 is able to induce antibodies to its components. It would be helpful to include unvaccinated age matched subjects in the study.

2.2.7 Question 7

Could the findings be explained by any non-specific antibody binding, or differences between Pandemrix and Arepanrix other than polysorbate 80 content?

Based on the evidence available to date, the specificity of the assay cannot be confirmed. It remains questionable whether the affinity of the described interactions is significant. The reaction observed by the investigators may plausibly represent non-specific low affinity binding of IgG antibodies to vaccine unrelated epitopes.

Based on the data submitted the assay cannot be considered specific or properly validated. The antigen suspension of Pandemrix differs from Arepanrix, and also contains polysorbate 80 and Triton X-100. Given that no other detergents are used in the immunoassay the presence of these detergents in the Pandemrix antigen component could explain the inhibition of binding of plasma samples from “high” binding individuals to AS03.

2.2.8 Question 8

Is there a scientific rationale for extrapolating the findings to any other influenza vaccines, including Fluarix?

The investigators hypothesise that polysorbate 80 in the H1N1 antigen suspension could be the factor which revealed novel epitopes in the Pandemrix vaccine and this response cross-reactive with self-antigen and enhanced by AS03 could have contributed to the development of narcolepsy. However, the current data do not lead to this conclusion. There is also no evidence to suggest that other vaccines containing polysorbate 80, including Fluarix, have been associated with an increased risk of narcolepsy.

The suggestion that other vaccines may be a risk factor for development of narcolepsy remains hypothetical and is not supported by the available data.

2.2.9 Question 9

Based on the data presented, is there a potential risk of narcolepsy when utilising a vaccine with a similar construct e.g. Fluarix, or influenza vaccines with similar adjuvant/excipients/residuals, in individuals previously and those not previously vaccinated with Pandemrix?

The data provided by the new study is preliminary and hypothesis generating. However, the results do not lead to the conclusion of specific differential immune response linked to a particular vaccine construct, nor to ascertain its contribution to the risk of narcolepsy.

Based on the available data, the role of the antigen, adjuvant or any of its constituents on the association between Pandemrix and narcolepsy remains unknown. There is no basis to extrapolate the findings to any other vaccine.

2.2.10 Question 10

If the Committee considers there to be an increased risk which could be linked to narcolepsy, I would also ask the Committee to indicate whether these concerns could be applicable to other vaccines (or other biologicals) and if they should be investigated further at Community level.

The data is not sufficiently validated or robust to provide any conclusions on a potential risk of narcolepsy at this point in time. There is no specific evidence base to extrapolate these findings to other vaccines.

3. Overall discussion

On 10 October 2012, the National Institute for Health and Welfare (THL) in Finland updated its recommendations on seasonal influenza vaccination within its national vaccination programme. These recommendations were based on a preliminary research report from THL that has suggested that there may be immunological differences between Arepanrix and Pandemrix. According to this research it could not be excluded that other H1N1 vaccines with similar viral antigens and method of production could trigger narcolepsy especially in children primed earlier with Pandemrix vaccination. The report suggested this may explain why the signal of narcolepsy has not been observed (so far) in Canada where Arepanrix was used, but has been confirmed in some countries in Europe where an association between vaccination with Pandemrix and narcolepsy in children and adolescents was observed. The report suggested that polysorbate 80 (Tween 80) in the H1N1 antigen suspension could be the factor which revealed novel epitopes in the Pandemrix vaccine and this response cross-reactive with self-antigen and enhanced by AS03 contributed to the development of narcolepsy. The CHMP was asked to give an opinion on questions regarding these new research findings.

The CHMP considered that the findings are at present preliminary. Based on the available information the specificity of the antibodies identified in these assays remains unknown.

It is possible that antibodies to AS03 in Pandemrix vaccinated individuals could be identified in controlled assays. Antibodies to a component of AS03 have been identified previously in plasma samples from both vaccinated and non-vaccinated subjects. However, there is no evidence that the antibodies identified in this study are specific for AS03 components or other vaccine components.

The role of the detected antibodies in the development of narcolepsy cannot be determined.

The available data do not support the assertion that the antibodies are specific to polysorbate 80. The data also do not allow any conclusion that they may be a factor in the development of narcolepsy. Based on the available data the role of polysorbate 80 contained in the antigen and/or adjuvant of Pandemrix in the development of narcolepsy has not been established.

There are several differences between Pandemrix and Arepanrix vaccines and it remains a plausible theory that differential antibody responses may be induced by the vaccines, that these assays may indicate the presence of such differential responses, and indeed that such differential immune response may be a factor in the development of narcolepsy. However, given the possibility that specificity cannot be confirmed, these assays cannot be considered as robust evidence of such an effect at this point in time.

Overall, the data do not provide robust evidence of an immunological basis for narcolepsy associated with Pandemrix, nor for concerns regarding other vaccines or products that contain polysorbate 80, nor for immunological differences versus Arepanrix.

There is no evidence to suggest that other vaccines containing polysorbate 80, including Fluarix, have been associated with an increased risk of narcolepsy. In this regard, any suggestion that other vaccines containing polysorbate 80 may be a risk factor for development of narcolepsy remains hypothetical and is not supported by the available data.

4. Overall conclusions

The Committee considered the procedure under Article 5(3) of Regulation (EC) No 726/2004 for immunological differences of pandemic vaccines initiated by the Executive Director of the European Medicines Agency.

The Committee concluded that the data presented are preliminary. As the biological mechanism through which Pandemrix vaccine has contributed to an increased risk of narcolepsy remains unknown, it cannot be excluded that there may be a specific differential immune response to Pandemrix and Arepanrix, which may have contributed to the risk of narcolepsy. However, based on the available information it cannot be concluded that this new research provides any evidence of this. The research remains hypothesis generating at this stage. The specificity of the antibodies identified in these assays remains unknown and therefore is no evidence of a specific differential immune response in the plasma samples of patients who developed narcolepsy and healthy vaccinated subjects who received Pandemrix. Similarly, the research provides no evidence that the identified antibody binding is specific to a polysorbate 80/H1N1 complex, nor that the identified antibodies have a role in the development of narcolepsy. The Committee considers that there is no basis on which to extrapolate the findings to any other vaccine, including Fluarix. At this point in time and based on the data available, the Committee considers that the findings do not support any regulatory action.

The Committee considers it important that the biological mechanism for the association between Pandemrix and narcolepsy is fully evaluated and the root cause identified. The marketing authorisation holder (MAH) for Pandemrix was previously requested by the CHMP to conduct studies to elucidate this, following its 2011 review of the benefit risk balance on Pandemrix and narcolepsy[4]. These obligations are reflected in Annex II of the Marketing Authorisation but results of this research programme are awaited. The MAH should take into account the current hypothesis generated by the THL research in its study programme. The Committee will fully evaluate the findings when available.

The Committee welcomes the work of academic research and appreciates that this is made available in a timely manner. However, at this point in time, the role of the Pandemrix antigen and its adjuvant on the association between Pandemrix and narcolepsy remains unknown. The Committee would welcome the opportunity to evaluate further analyses produced by these or other researchers as their work progresses.

[4] The CHMP's report on the benefit/risk balance review on Pandemrix and narcolepsy can be found at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000832/WC500118056.pdf