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Vaccine-induced antibody responses in relation to season. An analysis on Hepatitis B, Rubella and Measles

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Samenvatting

Het effect van seizoen op de vorming van antistoffen na vaccinatie tegen hepatitis B, mazelen en rubella werd onderzocht. Gezien de immunosuppressieve effecten van ultraviolette straling, met name de B-fractie (UVB), was de hypothese dat vaccinaties in de zomer gevolgd worden door relatief lage titers.

IgG bepalingen en datums van vaccinatie waren beschikbaar uit diverse bronnen: 1. uit een observationeel onderzoek naar de opbouw van immuniteit na vaccinatie tegen hepatitis B in een groep paramedische studenten in Utrecht, 2. uit een experimentele studie naar het effect van kunstmatige UVB belichting op de opbouw van immuniteit na vaccinatie tegen hepatitis B, 3. uit een transversaal sero-surveillance onderzoek waarbij in een random sample van de Nederlandse bevolking de antistoftiters tegen diverse vaccinaties bepaald werden. In de hepatitis B-onderzoeken werd de antistofvorming zoals gevolgd in de loop van een standaard vaccinatie protocol (Engerix -B, SB; 0, 1, 6 maand) naar seizoen van eerste vaccin injectie geanalyseerd. In het sero-surveillance onderzoek werden de anti-mazelen en anti-rubella antistoftiters in kinderen in het leeftijdstraject 2-7 jaar naar seizoen van eerste vaccin injectie en leeftijd geanalyseerd. Deze kinderen hadden op het moment van bloedafname één keer hun bof-, mazelen-, rubella vaccinatie (BMR) op de leeftijd van 14 maanden ontvangen.

De gegevens uit het onderzoek onder paramedische studenten lieten een licht vertraagde antistofvorming zien gedurende vaccinaties die in een zonnig seizoen waren begonnen. Echter, een seizoensverschil consistent in de loop van meerdere kalenderjaren werd niet gezien en aan het eind van het vaccinatie protocol waren er geen seizoensverschillen in mate van protectie. In de andere onderzoeken werden geen seizoensverschillen in antistoftiters gevonden.

Deze gegevens ondersteunen de hypothese van verminderde immunoprotectie door een hoog niveau van UVB blootstelling in de zomer slechts ten dele. In een fijnmaziger onderzoek, waarin rekening wordt gehouden met verschillen in persoonlijke blootstelling en gevoeligheid voor UVB, zouden eventuele subtiele effecten wellicht duidelijker aan het licht kunnen treden. Een advies voor de algemene bevolking om vaccinaties in een zonnig seizoen te vermijden, is op grond van deze bevindingen prematuur.

Summary

The effect of season on the antibody response after Hepatitis B (HB), Measles and Rubella vaccination in humans was investigated. In view of the immunosuppressive effects of ultraviolet radiation (UVR), especially the B-waveband (UVB), it was hypothesised that a lower antibody response after vaccination procedures that were started in summer might be detectable.

IgG assessments and dates of vaccination were available 1. from a survey on the formation of anti-HB antibodies (anti-HBs) after vaccination to HB among paramedical students in Utrecht, 2. from an experimental study on the effect of artificial UVB on the immune response to HB vaccine, and 3. from a cross-sectional serosurveillance study on various vaccinations in a random sample of the Dutch population. In the surveys on HB the antibody formation in the course of a standard HB vaccination procedure (Engerix-B, SB; standard 0, 1, 6 months) was analysed by season of first vaccine injection. In the serosurveillance study the anti-Measles and anti-Rubella IgG titers in children who were aged 2-7 years at the time of the survey, and who had received their Mumps-, Measles-, Rubella-vaccination only once (MMR-1, at 14 months of age) were analysed by age and season.

The data from the survey among paramedical students indicated a slightly retarded antibody response during vaccinations that were started in a sunny season. However, this finding was not consistently found after breaking down the data by calendar year and at the end of the procedure equal levels of anti-HBs were found. In the other surveys no seasonal influences on the formation of antibodies could be established.

These data support the hypothesis of a reduced immunoprotection due to high ambient UVR during sunny season only to a limited extent. The study design may have been too crude with respect to both personal differences in exposure and susceptibility to UVR and the immune responses following immunisation for demonstrating the postulated effect of UVR. An advice for the general population to avoid the starting of a vaccination procedure during sunny season appears to be premature at present.

1. Introduction

Humans are exposed to a variety of chemical, therapeutic and physical agents that may modulate the immune system. Studies in laboratory animals have shown that many environmental factors exert immunotoxic activity, including effects on the susceptibility to infections (1). A few studies have indicated that environmental factors that exert immunotoxic activity may increase the susceptibility to infections in humans (2, 3). However, in more general it is less well known whether immunotoxicity induced by environmental agents will have consequences for the susceptibility to infections in humans (1, 4). For example, experimental animal data show that ultraviolet radiation (UVR, 280-400 nm), especially the B-waveband (UVB), at doses relevant to outdoor exposure may affect specific cellular T-cell immune responses and increase the susceptibility to viral, bacterial and parasitic pathogens (5, 6). Effects of artificial UVR and sunlight on immune parameters have also been shown in humans (7, 8, 9, 10, 11). However, it is still an unresolved issue whether increases in UVR exposure due to ozone depletion and sun seeking behaviour are associated with increasing incidences of infectious diseases in the human population (12, 13).

Measurement of vaccine responses in terms of antibody titers has been recommended as a great opportunity to monitor the suppression of immune function due to immunotoxic influences on the population level (4, 12, 14). In experimental studies in rodents it has been shown that the antibody response to sheep erytrocytes is an adequate indicator for immunotoxicity, as major components of the immune system are involved in this humoral immune response. In addition, alterations in the response to sheep erythrocytes appeared to correlate well with the resistance to experimental infectious agents in these animal studies (15, 16). The advantage of using antibody responses to vaccination as readout in humans is that the effect of immunotoxic agents on the protection against an infection may be ascertained without the need to induce infections experimentally or to wait for actual infections to develop (12, 17). For Hepatitis B, Measles, and Rubella vaccination in humans the level of antibody formation appeared to be associated with protection to disease on exposure to the virus (18, 19, 20, 21). However, it is not possible for all vaccination responses in humans to extrapolate the vigour of the antibody response to protection. Even if an effect of exposure to an immunotoxicant on vaccination titers does not necessarily indicate a decreased protection to the pathogen at which the vaccination was aimed, these titers may still serve as a model of effects of exposures on immune responses that are required for protection to other infections (4). Immunotoxic effects on antibody formation has been described previously for cyclosporine (22), smoking (23), PCBs and dioxins (3).

Animal data show that UVR may reduce the antibody response following immunisation with a T cell-dependent antigen (24, 25). It was demonstrated in humans that short term exposure to UVR from solarium treatment or from sunbathing may cause a decrease in the CD4+/CD8+ T- cell ratio, and an increase in the suppressor T cell activity leading to impaired IgG production in vitro (7, 8). For this reason it can be hypothesised that vaccinations given in summer are associated with lower immune responses than vaccinations given in winter. Cellular immune responses to the vaccine precede the formation of antibodies and these responses are induced immediately after the first vaccination. As UVR exerts a short-term effect on

the cellular immunity, we regarded the season in which the first vaccine of a immunisation procedure was given as indicative of the exposure to UVR that might be of relevance for the formation of (protective) antibodies. For Hepatitis B, Rubella, and Measles we analysed pre-existing data on antibody formation and examined whether a seasonal influence actually could be established.

2. Methods

2.1 Hepatitis B - students paramedical college, Utrecht 1997-2000

2.1.1 Study population

Data on antibody responses during and after a Hepatitis B (HB) vaccination procedure were available from 522 students of two paramedical colleges in Utrecht, the Netherlands (nurses, dental hygienists, speech trainers). These students received their first dose recombinant HB surface antigen (HBsAg) vaccine (Engerix-B, GSK, 20μg) in January 1994 (n=25), October 1995 (n=24), February 1996 (n=19), September 1996 (n=77), August 1997 (n=68), September 1997 (n=2), April 1998 (n=33), August 1998 (n=19), January 1999 (n=19), May 1999 (n=20), June 1999 (n=1), September 1999 (n=54), October 1999 (n=27), January 2000 (n=22), February 2000 (n=2), or March 2000 (n=110). A standard immunisation procedure was applied with 3 vaccinations (I: 0, II: month 1, III: month 6) and with injection in the deltoid muscle. The young and healthy students were predominantly female and 17-20 years of age. All participants gave written informed consent.

2.1.2 Laboratory test

Serological assessment was done in blood taken at the time of the first vaccination, 3 and 7 days thereafter, at the time of the second vaccination, 3, 7 and/ or 14 or 30 days thereafter, at the time of the third vaccination and 1 month thereafter. The concentration of IgG antibody to HB surface antigen (anti-HBs) was assessed by means of an enzyme-linked immunosorbent assay (AUSAB, Abbott, Chicago, USA), performed on the IMx. An anti-HBs of $\geq$1000 IU/l was registered as '1000', which implies that evaluation of differences in the highest levels was limited. The cut-off values for protection were assumed to be 10 IU/l (level of low protection), 100 IU/l (level of high protection), and 1000 IU/l (level of high and lasting protection).

2.1.3 Statistical analysis

The geometric mean titer (GMT, ^{10}log-transformation) and 95%-Confidence Interval (95%-CI) were calculated by quarter of first vaccine and calendar year and for every point of time of blood sampling in the course of the immunisation procedure separately. We considered subjects who started the vaccination procedure in spring or summer (quarter 2: April through June, quarter 3: July through September) to be exposed to relatively high doses of ambient UVR at the time of the first vaccine (n=274). We considered persons who started the vaccination procedure in autumn or winter (quarter 1: January through March, quarter 4: October through December) to be exposed to low doses of ambient UVR at the time of the first vaccine (n=248). Furthermore, the percentage of participants with an anti-HBs at the cut-off level or higher was calculated, again by season of first vaccine and calendar year. The cut-off level was chosen according to the stage of the immunisation procedure: 10 IU/l (3, 7 days after vaccination I, at the time of vaccination II and 3 days thereafter), 100 IU/l

(7, 14, and 30 days after vaccination II), 1000 IU/l (at the time of vaccination III and at day '7 month'.
The difference in antibody formation between vaccinations that were started in 'winter' (quarter 1 and 4) and vaccinations that were started in 'summer' (quarter 2 and 3) without a distinction by calendar year of first vaccination was also calculated to get an overall impression. This crude difference by season ('winter' versus 'summer') for every point of time during the immunisation procedure separately was tested by means of the non-parametric one-way-Wilcoxon test (GMT) and the χ^2 test for categorised data (percentage).

2.2 Hepatitis B – trial for effect of artificial UVB, University Medical Centre Utrecht 1998-2000

2.2.1 Study population

A trial was performed to examine the effects of experimental exposure to UVR on the immune responses to hepatitis B vaccine. Healthy volunteers, both male and female, and predominantly aged 19-21 years were recruited from the University Medical Centre (n=191). A part of the volunteers (n=97) received their personal Minimal Erythema Dose of broadband UVB (total body) on 5 days in the week prior to the first vaccination (cabinet with Philips TL12 lamps: 280-315 nm). The other volunteers comprised the control group (n=94). A standard vaccination procedure with recombinant HBsAg vaccine (Engerix-B, SB, 20µg) was applied (intramuscular vaccination; 0, 1, 6 month). The participants started their immunisation procedure in November 1998 (n=34), January 1999 (n=31), February 1999 (n=35), October 1999 (n=14), in November 1999 (n=18), January 2000 (n=42), or February 2000 (n=17). Each volunteer was fully informed and gave written informed consent before entry into the study. The trial will be described in detail elsewhere.

2.2.2 Laboratory test

Blood was sampled from all participants before vaccination (day –11 and day 0), two weeks thereafter (day 14), at the time of the second vaccination (day 32), 3, 6, 14 days, and 1 month thereafter (day 35, 38, 46, and 60), and 1 month after the third vaccination (day '7 month'). At these points of time the anti-HBs was measured. The quantitative determination of anti-HBs was performed by Microparticle Enzyme Immunoassay (MEIA) using Axsym (Abbott, Chicago, USA).
Furthermore, at day –11, day 0, day 14 and day 38 peripheral blood mononuclear cells (PBMC) were obtained by gradient centrifugation and lymphocyte stimulation tests were performed. For antigen-specific stimulation this was done by culturing the PBMC at $4 * 10^5$ cells/ well in triplicate (37^0C) and adding HBsAg in different final concentrations. After 5 days incubation 10 µl (1 µCi) of ^{3}H-Thymidine was added and incubated for an additional 20-22 hours. The activity of the cells was counted in a liquid scintillation counter (LKB-Wallac). The antigen specific lymphocyte proliferation response is presented as the difference between the number of counts per minute due to ^{3}H-Thymidine incorporation (cpm) in the presence of HBsAg and the cpm in the absence of HBsAg (delta-cpm).

2.2.3 Statistical analysis

The GMT (^{10}log-transformation) and 95%-CI were calculated by season of first vaccine (autumn versus winter), experimental exposure to UVR (yes/ no) and for every point of time of blood sampling in the course of the immunisation procedure separately. For the present analysis we assumed that participants who received their first vaccine in autumn 1998 or autumn 1999 were exposed to relatively higher levels of ambient UVR at that time than participants who started their vaccination procedure in winter 1999 or winter 2000. The analysis was performed for the experimental and control group separately to exclude the possible confounding effect of experimental exposure to UVB. Furthermore, the percentage of participants with an anti-HBs at the cut-off level or higher was calculated by quarter of first vaccine, experimental exposure and point of time. The cut-off levels were chosen as follows: 10 IU/l for days 0-38, 100 IU/l for day 46 and 60, 1000 IU/l for day '7 month'. The mean lymphocyte proliferation response to HBsAg (Δcpm) and 95%-CI were also calculated by season of first vaccine, experimental exposure and for the four above mentioned points of time (days -11, 0, 14, 38) separately.

2.3 Measles – National Serosurveillance Study

2.3.1 Study population

Data for our analysis were obtained from a cross-sectional population-based serosurveillance study, which was carried out in the Netherlands in 1995/ 1996. Within 40 municipalities an age-stratified sample of 380 persons was drawn. Eligible individuals were asked to fill out a questionnaire on various socio-demographic characteristics, to give a blood sample and to bring vaccination certificates from the National Immunisation Programme. The study has been described in detail elsewhere (26, 27). For the present study the anti-Measles antibodies in the sera of 719 participating children aged 2-7 year were analysed by season of first vaccination. These children had a documented vaccination history for the combined Measles, Mumps, Rubella vaccine (MMR). As MMR is given at the age of 14 months (MMR-1) and in the year the child becomes 9 years old (MMR-2), these children had received the vaccine only once at the time of the blood sampling.

2.3.2 Laboratory test

The concentration of IgG antibody against purified measles virus (anti-Measles IgG) was measured in the serum samples by an ELISA, which has been described in essence elsewhere (27, 28). Titers below the minimum level of detection of 0.01 were set to 0.005 IU/l to enable log-transformation (see below). An anti-Measles IgG higher than or equal to the cut-off level of 0.2 IU/l was assumed to correlate with protection, which is a widely accepted value (19, 27).

2.3.3 Statistical analysis

The GMT (^{2}log-transformation) and 95%-CI were calculated by age of blood sampling and season of first vaccine. Season was the quarter of the year in which the child was vaccinated with the MMR-1. Furthermore, the percentage of children with an anti-Measles IgG at the level of protection or higher was calculated, again by age of blood sampling and season of first vaccine.

2.4 Rubella – National Serosurveillance Study

2.4.1 Study population

In the same sera (see **2.3.1.**) the anti-Rubella antibodies were tested and analysed by age of blood sampling and season of first vaccination. The sera of 718 children aged 2-7 years were included in the analysis. Again, all participating children included in the present analysis had received the Rubella vaccine only once at the time of blood sampling.

2.4.2 Laboratory test

The concentration of IgG antibody against Rubella virus (anti-Rubella IgG) was measured in the serum samples by a standard ELISA (28, 29). The minimum level of detection was 1.0 IU/ml. The cut-off value for protection was assumed to be 10 IU/ml, which is in agreement with international standards (21, 29).

2.4.3 Statistical analysis

The GMT (^{e}log-transformation) and 95%-CI were calculated by age of blood sampling and season of first vaccine. Season was the quarter of the year in which the child was vaccinated with the MMR-1. Furthermore, the percentage of children with an anti-Rubella IgG at the level of protection or higher was calculated, again by age of blood sampling and season of first vaccine.

3. Results

3.1 Hepatitis B - students paramedical college, Utrecht 1997-2000

3.1.1 Mean titers

In *figure 1* the mean anti-HBs (GMT) is given by season, calendar year and day of blood sampling. No anti-HBs could be detected shortly after the first vaccination (data not shown). From the second vaccination onwards a clear rise in the GMTs is observed. At the end of the immunisation procedure (day '7 month') GMTs next to 1000 IU/l were found.
At the time of the second vaccination (day 30), 3 days (day 33) and 30 days (day 60) later, and at the time of the third vaccine injection (day '6 month') a tendency towards a higher GMT was found when the first vaccine was received in the first or fourth quarter of the year. At day 60 and day '6 month' these differences reached the level of statistical significance. However, this tendency was not consistently found after breaking down the data by calendar year of vaccination. Irrespective of season both comparatively high and low GMTs were found at the different points of time during the immunisation procedure. Furthermore, at the end of the immunisation procedure (day '7 month') a higher GMT was found when the first vaccine was received in a season of relatively high ambient UVR (quarter 2 and 3).

3.1.2 Percentage of persons with protective levels

In *figure 2* the percentage of participants with an anti-HBs at the appropriate cut-off level or higher is given by season, calendar year and day of blood sampling. Up to the second vaccination (day 30) hardly any participant reached an anti-HBs $\geq$10 IU/l (data not shown). From the time of the second vaccination onwards a considerable number of participants reached protective levels of anti-HBs ($\geq$10 IU/l).
There was a tendency towards a higher percentage of participants with a low and where appropriate high protective level of anti-HBs ($\geq$10 IU/l and $\geq$100 IU/l respectively) at the different points of time in the course of the immunisation procedure when the first vaccine injection was administered in the first or fourth quarter of the year (no distinction by calendar year). However, this tendency was not consistently found after breaking down the data by calendar year of vaccination. Irrespective of season both comparatively high and low percentages of persons with protective levels were found at the different points of time during the immunisation procedure. Furthermore, at the end of the immunisation procedure (day '7 month') a higher percentage of participants with an antiHBs $\geq$1000 IU/l was found when the first vaccination was received in the second or third quarter of the year (89.1% vs. 86.2%). At the end of the immunisation procedure (day '7 month') no seasonal difference in percentage of participants with an anti-HBs $\geq$100 IU/l (level of sufficient protection) was observed (winter: 98.5%, summer: 97.1%, p=0.30; data not shown).

In conclusion, a tendency towards a comparatively low mean anti-HBs after vaccinations that were started in spring or summer was found when the data were analysed by season only. However, this finding could not be established consistently

when the data were broken down by both season and calendar year of first vaccination. At the end of the procedure there was no significant seasonal difference in level of protection.

3.2 Hepatitis B – trial for effect of artificial UVB, University Medical Centre Utrecht 1998-2000

3.2.1 Mean titers

In *figure 3* the mean anti-HBs (GMT) is given by day of blood sampling, season, and experimental exposure. Shortly after the first vaccination (day 14) hardly any antibodies were detectable. From the second vaccination onwards a clear rise in the GMTs was observed. At the end of the immunisation procedure (day '7 month') GMTs next to 1000 IU/L were measured. No statistically significant differences in the GMTs related to season of first vaccination or experimental exposure were found when considering the data by point of time separately. At day 38, day 60 and day '7 month' a non-significant tendency towards a higher anti-HBs was observed in the group of participants who received their first vaccine during autumn.

3.2.2 Percentage of persons with protective levels

In *figure 4* the percentage of participants with an anti-HBs at the appropriate cut-off level or higher is given by day of blood sampling, season, and experimental exposure. Up to day 35 hardly any participant reached an anti-HBs of 10 IU/l or higher. From day 38 onwards (6 days after the second vaccination) considerable numbers of participants reached protective levels of anti-HBs (>=10 IU/l). No statistically significant differences by season or experimental exposure could be established. At day 38, day 46, day 60, and day '7 month' a statistically non-significant tendency towards a higher percentage with an anti-HBs at the appropriate cut-off level or higher was observed in the group of participants who received their first vaccine during autumn. At day '7 month'100% of the participants who started their vaccination procedure in autumn and 92% of the participants who started their vaccination in winter had reached anti-HBs $\geq$100 IU/l (level of sufficient protection).

3.2.3 Lymphocyte proliferation responses to HBsAg

In *figure 5* the mean lymphocyte proliferation response to HBsAg is given by day of blood sampling, season and experimental exposure. Before (day –11) and at the time of the first vaccination (day 0) hardly any response to HBsAg could be measured. At day 14 after the first vaccination and day 6 after the second vaccination (day 38) considerable lymphocyte proliferation response to HBsAg was measured. Receiving the first vaccine in winter was associated with a comparatively high proliferation. At day 38 the seasonal difference reached the level of statistical significance.
No effects of artificial UV exposure were found.
In addition, no statistically significant differences in anti-HBs by season of first vaccine injection could be established. However, vaccinations that were started in winter were associated with higher antigen-specific lymphocyte proliferation responses than vaccinations started in autumn. This finding may indicate a seasonal fluctuation.

3.3 Measles – National Serosurveillance Study

3.3.1 Mean titers

In *figure 6* the mean anti-Measles IgG (GMT) is shown by season of vaccination and age of blood sampling. A clear tendency towards lower GMTs with increasing age and thus with time of vaccination was observed. No differences that might indicate a seasonal fluctuation could be established.

3.3.2 Percentage of children with protective levels

In *figure 7* the percentage of children with a protective level of anti-Measles IgG is shown by season of vaccination and age of blood sampling. A tendency towards a lower percentage with increasing age was observed. In the group of children at the age of 7 who were vaccinated during spring or summer the percentages with a protective level of anti-Measles IgG were higher than those in the group of children at the age of 7 who were vaccinated during autumn or winter. However, the differences did not reach the level of statistical significance. At the other ages no differences that might indicate a consistent seasonal fluctuation could be established.

In conclusion, no seasonal difference in the anti-Measles IgG after vaccination could be established. Lower anti-Measles IgG concentrations with increasing age were found.

3.4 Rubella – National Serosurveillance Study

3.4.1 Mean titers

In *figure 8* the mean anti-Rubella IgG (GMT) is shown by season of vaccination and age of blood sampling. A clear tendency towards lower GMTs with increasing age was observed. At the ages of 4 and 7 years lower GMTs were found when vaccinated in the sunny season (quarter 2 or quarter 3). The seasonal differences, however, did not reach the level of statistical significance.

3.4.2 Percentage of children with protective levels

In *figure 9* the percentage of children with a protective level of anti-Rubella IgG is shown by season of vaccination and age of blood sampling. A tendency towards a lower percentage with increasing age was observed. At the ages of 5 and 7 it was shown that in the group of children who were vaccinated during spring or summer the percentages of protective antibodies were lower than those in the group of children who were vaccinated during autumn or winter.
However, the differences did not reach the level of statistical significance. At the other ages no consistent seasonal differences were found.

In conclusion, no seasonal differences in the anti-Rubella IgG concentration after vaccination could be established. Lower anti-Rubella IgG concentrations with increasing age were found.

4. Discussion

In the present study it was investigated whether a seasonal influence on the formation of antibodies after vaccination to Hepatitis B, Measles or Rubella could be established. It was hypothesised that a sunny season might be associated with a less favourable antibody formation. This hypothesis was based on the assumption that UVR causes suppression of cellular immune functions that underlie the formation of protective antibodies. It has been postulated that UVR causes a shift from a T helper-1 (Th-1) to a T helper-2 cytokine profile (Th-2) (30). This might imply that UVR may induce an enhancement of those antibody responses that are Th-2 mediated and simultaneously suppress some Th-1 dependent cellular defence mechanisms (31). However, it was shown in mice that UVR inhibits not only Th-1 associated isotypes (IgG2a), but also Th-2 associated isotypes (IgG1, IgE) (24, 25, 32). A seasonal influence on immune responses due to UVR might be observed especially in those countries that experience a very strong seasonal fluctuation in ambient UVR like Northern-European countries (33). The establishment of this seasonal influence, if any, is important as this should indicate immunosuppression and as a consequence increased susceptibility to infections due to exposure to UVR in human populations.

In this study no clear and consistent influence of sunny season, either favourable or deleterious, on the antibody response after vaccination was found. In the survey among paramedical students the data indicated a slightly retarded immune response when the first vaccine was given in a sunny season. However, this did not appear to be of clinical importance as this seasonal difference could not be established consistently in the course of the immunisation procedure and even a (non-significantly) higher level of protective antibodies was found at the end of the procedure in vaccinations that were started in a sunny season (*figure 1 and 2)*. Furthermore, no differences that might indicate an influence of sunny season on the antibody formation could be established in the trial on the effect of artificial UVB on anti-HBs (*figure 3 and 4*), the sero-survey on anti-Measles IgG (*figure 6 and 7*), and the sero-survey on anti-Rubella IgG (*figure 8 and 9*). In the trial on the effect of artificial UVR on anti-HBs a small but non-statistically significant tendency towards a higher percentage of persons with a protective level of anti-HBs was found when starting the vaccination in autumn in comparison with vaccinations that were started in winter. On the contrary, the mean lymphocyte proliferation response to HBsAg, which indicates the cellular immune response to the vaccine, appeared to be statistically significantly higher in persons who started their vaccination in winter in comparison with persons who started their vaccination in autumn (*figure 5*). This finding itself is in accordance with our hypothesis of suppressed cellular immunity during periods of relatively high levels of ambient UVR (e.g. autumn versus winter). As the contrast in ambient UVR in October or November and the ambient UVR in January or February is comparatively small, we need more seasonal data to verify and interpret this result. Assuming that this finding is due to the effect of ambient UVR, it might indicate that different arms of the human immune system are differentially influenced (31). The results from the present analysis do not support the hypothesis of impaired antibody formation due to high levels of ambient UVR during sunny season. It may be questioned whether studying seasonal influences are suitable for human studies on the effect of UVR on immunological parameters. Sunny season is associated with high levels of ambient UVR, but also with higher ambient temperature and longer days. It has been reported that temperature and photoperiod also influences the immune system (34, 35). Furthermore, yearly rhythms in immunological

parameters that are independent of environmental influences have been described (36, 37). It is hard to disentangle the effects of different factors that have a seasonal association in common. A study design in which season is regarded as a confounder and in which small fluctuations in exposure to solar UVR due to the effect of clouding or to the effect of differences in outdoor behaviour are measured (ambient and personal monitoring respectively) should be helpful for this purpose.

It may be argued that the postulated effects of UVR are subtle and possibly less pronounced in comparison with other factors that influence the immune system along the yearly scale. This implies that the possibly subtle immunosuppressive effect of UVR is hard to establish from a bird's- eye view as we tried by searching after this effect in the human population as a whole. Differences in susceptibility to UVR induced immunosuppression like differences in sensitivity to sunburn exist in humans (38). This means that we need to take differences in susceptibility to UVR into account in human studies on the effects of UVR on the human immune system. A subgroup of humans who are fully susceptible for the deleterious effects of UVR may be characterised. Furthermore, the immunosuppressive effects of UVR in humans may appear only at high personal doses that are associated with local erythema or sunburn (39). This means that in our study a suppressive effect on the antibody formation might be measurable only in persons who suffer from erythema or even sunburn at the time of their vaccination. This implies that the season of the year is a very rough measure for the personal exposure at the time of vaccination and that personal differences in exposure at a more detailed level have to be taken into account.

From the presented data it can not be concluded that vaccinations given in sunny season are associated with a less favourable formation of protective antibodies than vaccinations given in winter. Therefore, an advice for the general population to avoid the starting of a vaccination procedure in summer appears to be premature. For future research we recommend a study in which the immunomodulating effects of UVR on the immune response are examined in more detail, both with respect to the cellular mechanisms underlying the antibody formation, the personal exposure to sunlight, and differences in susceptibility to UVR. Such a study will be helpful to judge whether the present conclusion also applies to the immune responses underlying the antibody formation, and to a subgroup of humans who are highly sensitive for UVR or who are highly exposed to sunlight at the time of their vaccination.

Appendix 1 Figures

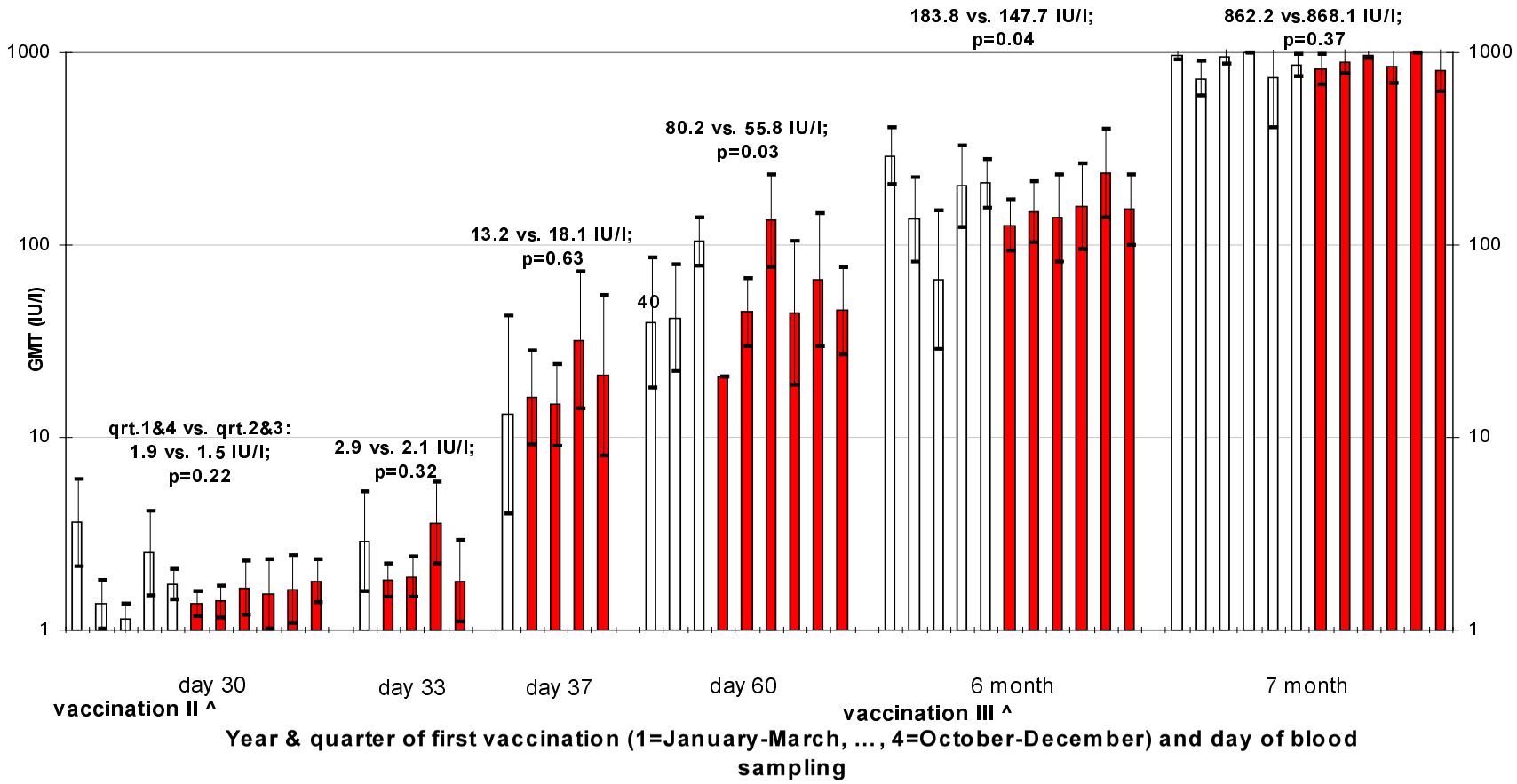

Figure 1. GMT of anti-HBS (IU/l) and 95%-CI in the course of the immunisation procedure by year and season of first vaccine.

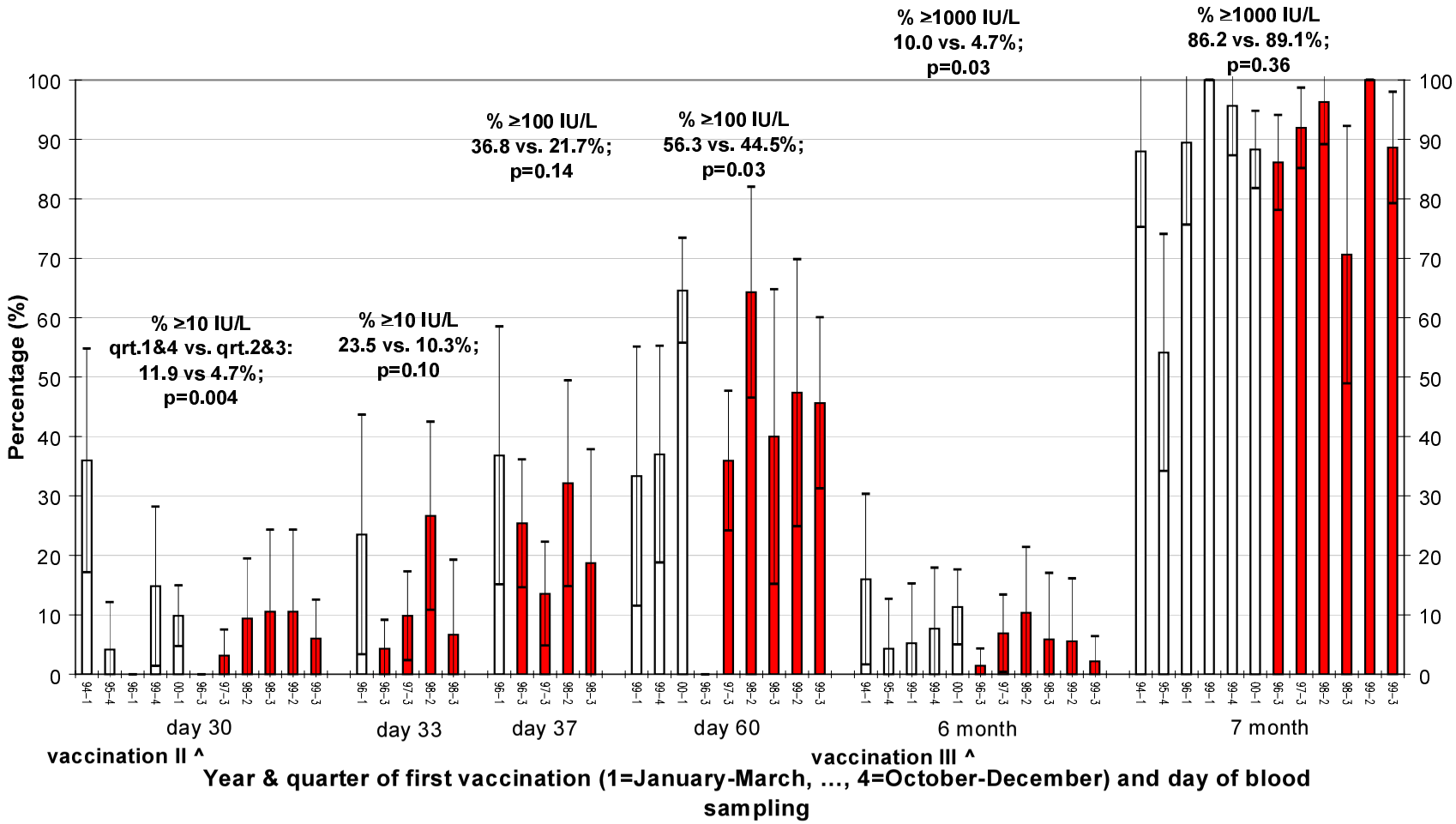

Figure 2. Percentage of participants with an anti-HBs at the appropriate cut-off level or higher (</≥10, </≥ 100, </≥ 1000 IU/l) and 95%-CI in the course of the immunisation procedure by year and season of first vaccine.

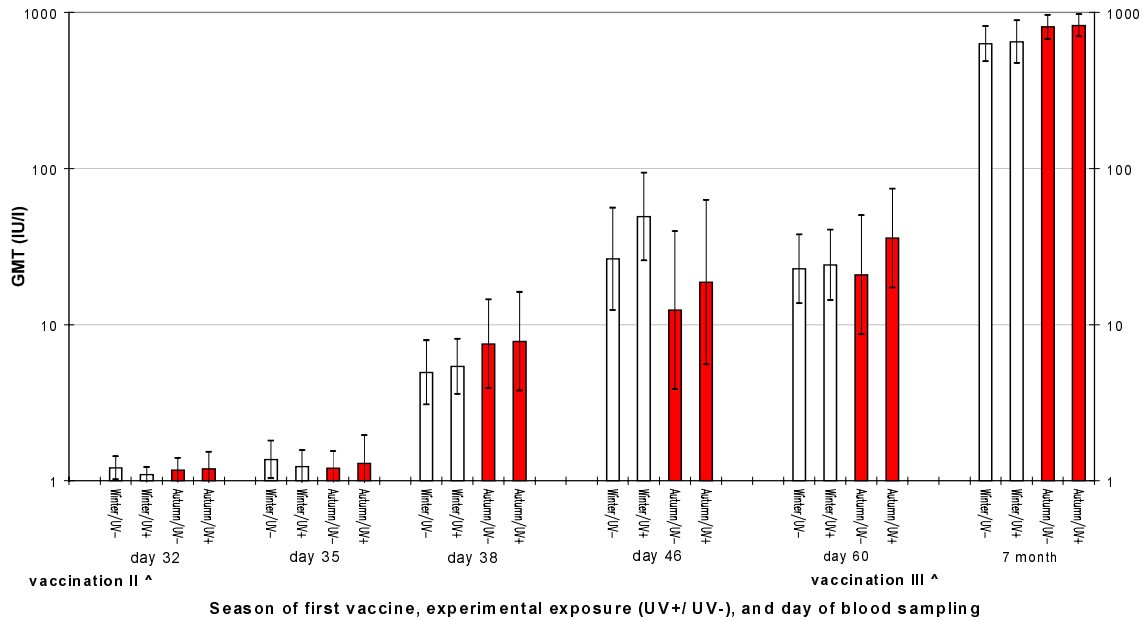

Figure 3. GMT of anti-HBS (IU/l) and 95%-CI in the course of the immunisation procedure by season of first vaccine and experimental exposure to UVR (Philips TL-12).

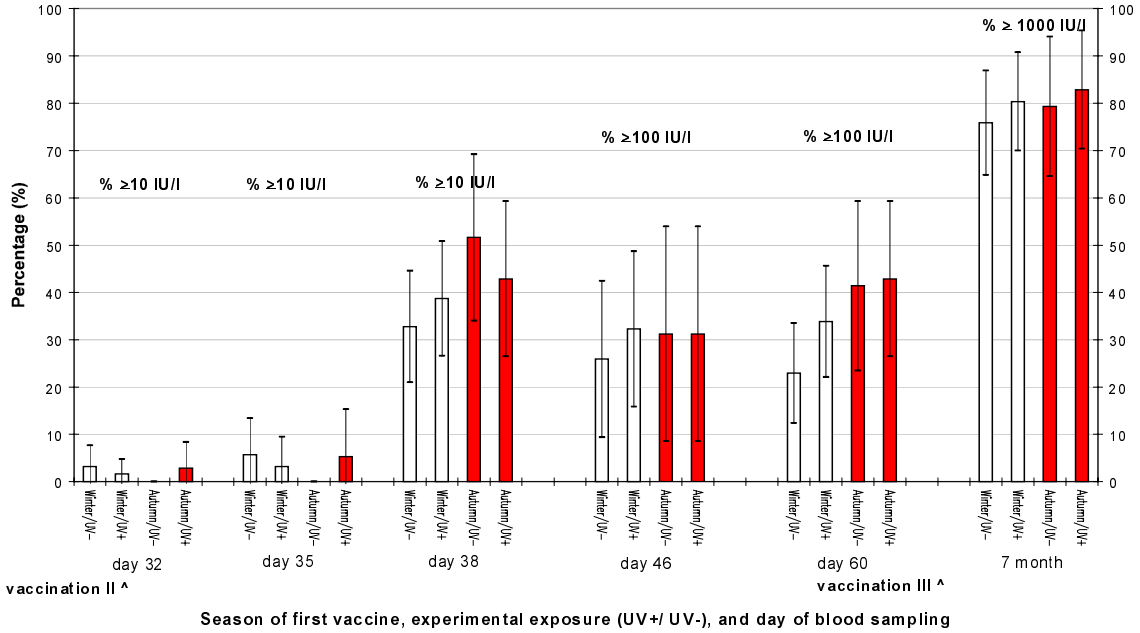

Figure 4. Percentage of participants with an anti-HBs at the appropriate cut-off level or higher (</≥10, </≥ 100, </≥ 1000 IU/l) and 95%-CI in the course of the immunisation procedure by season of first vaccine and experimental exposure to UVR (Philips TL-12).

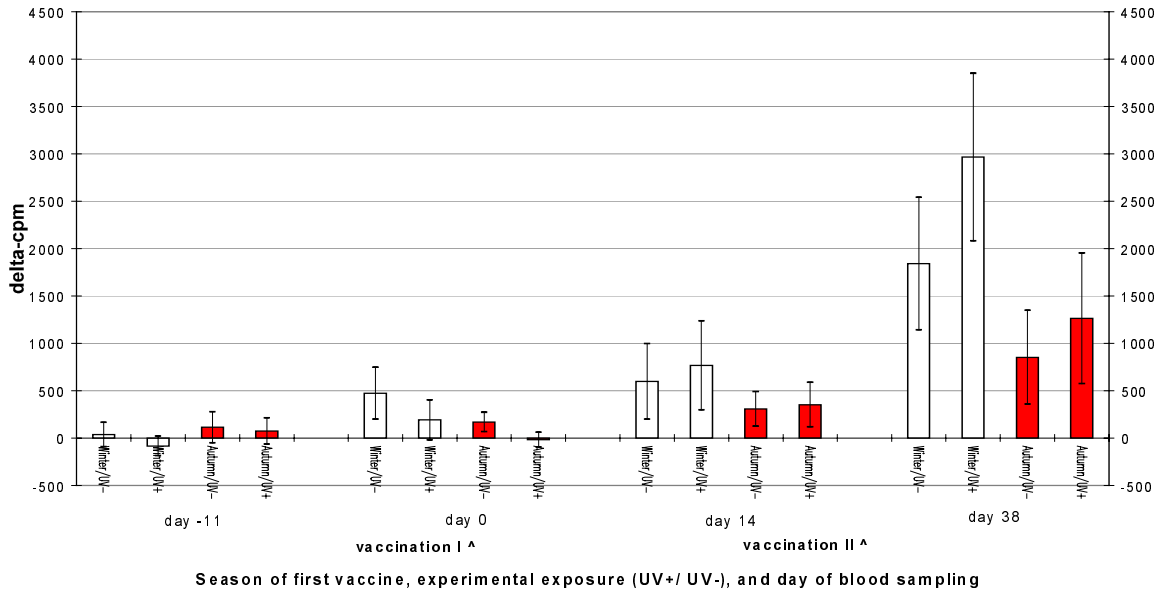

Figure 5. Mean lymphocyte proliferation response to HBsAg and 95%-CI in the course of the immunisation procedure by season of first vaccine and experimental exposure to UVB (Philips TL-12).

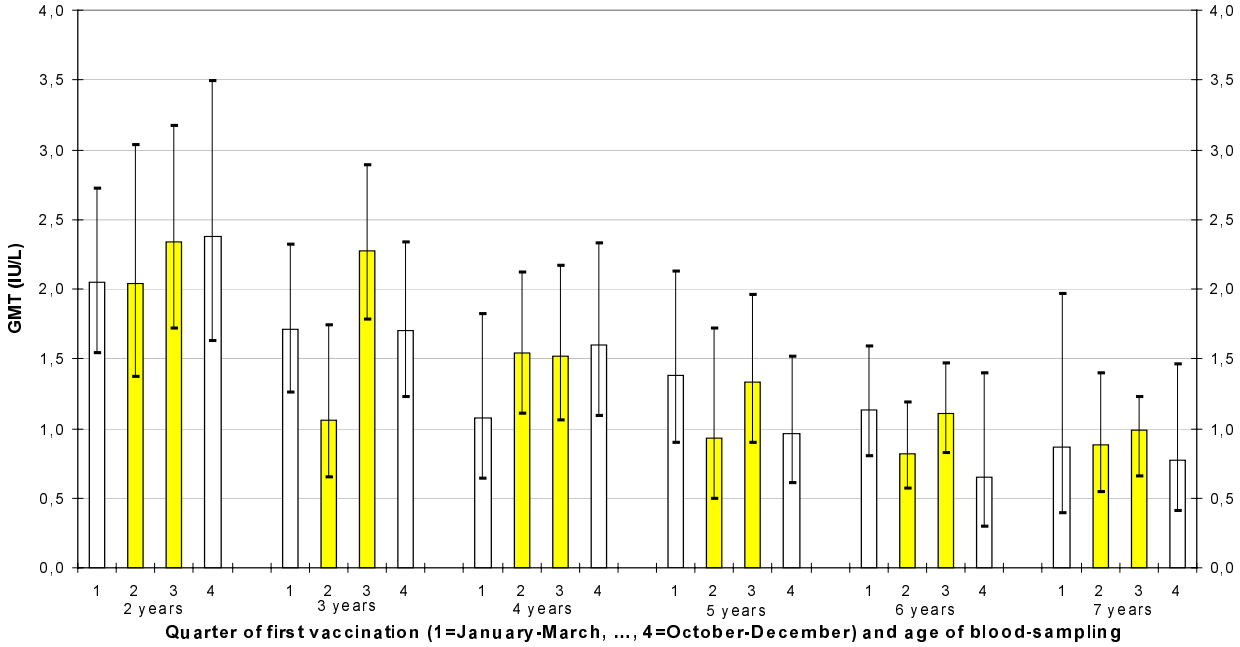

Figure 6. GMT of anti-Measles IgG (IU/l) and 95%-CI by season of first vaccine (MMR-1) and age of blood sampling.

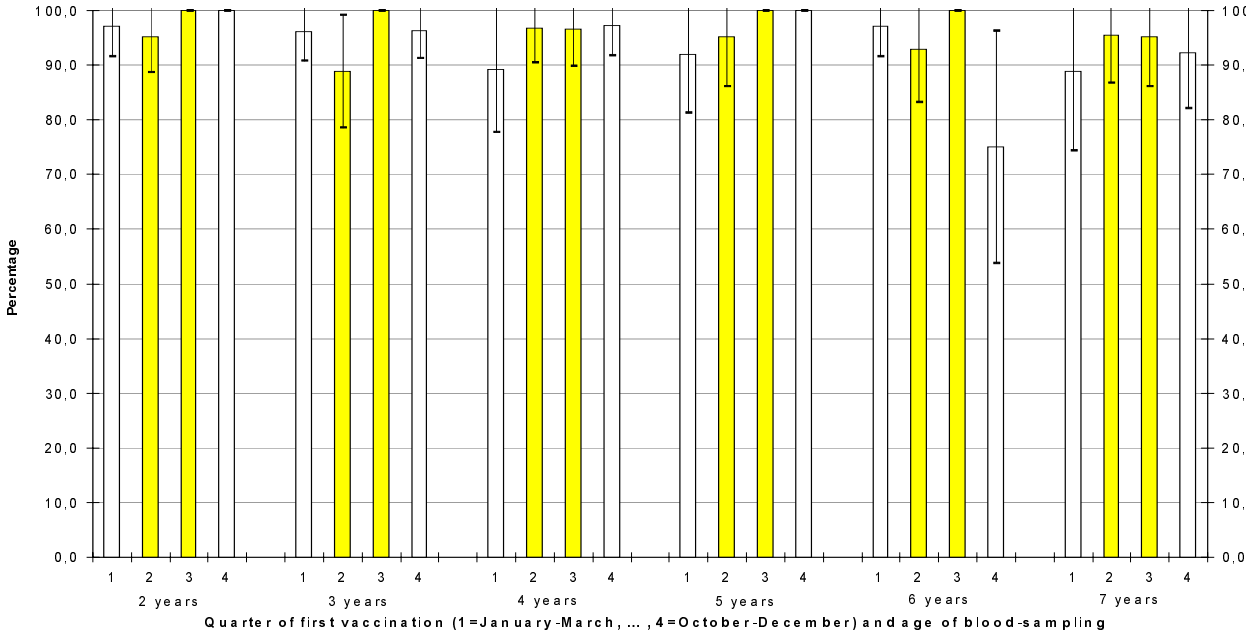

Figure 7. Percentage of children with an anti-Measles IgG at the cut-off level or higher (</≥ 0.2 IU/l) and 95%-CI by season of first vaccine (MMR-1) and age of blood sampling.

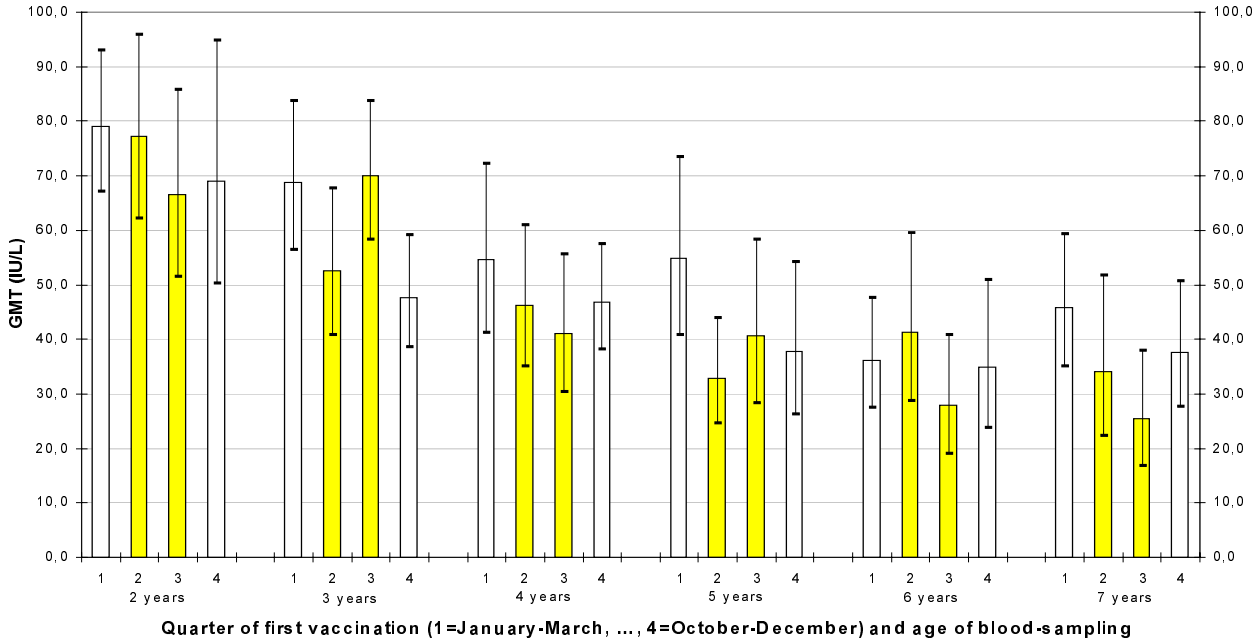

Figure 8. GMT of anti-Rubella IgG (IU/l) and 95%-CI by season of first vaccine (MMR-1) and age of blood sampling.

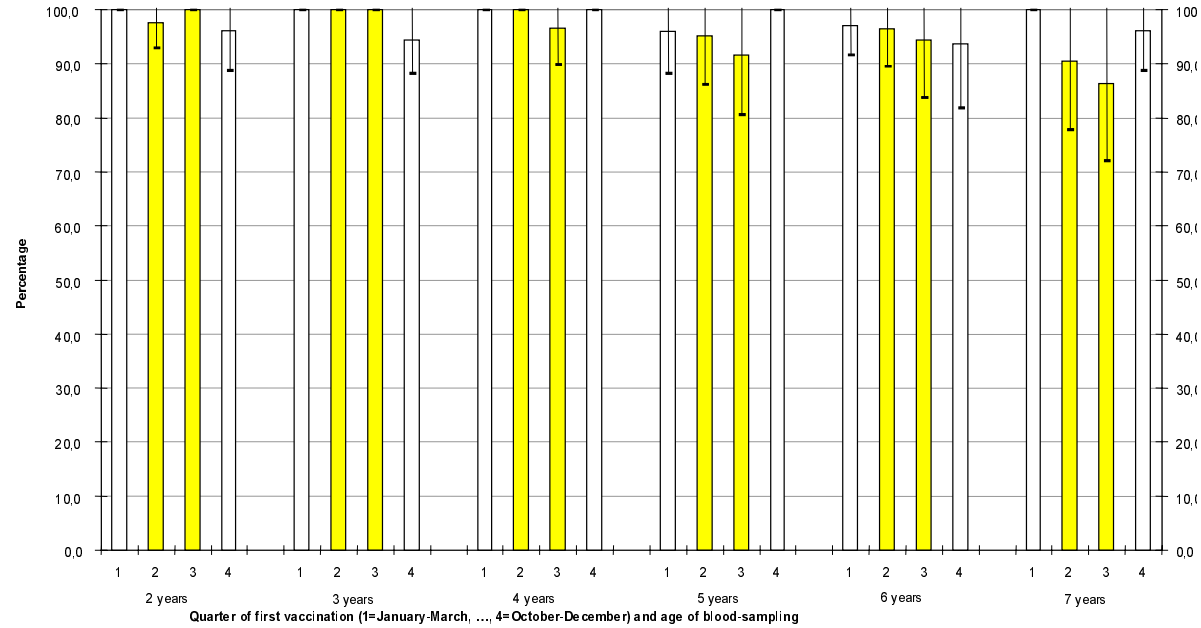

Figure 9. Percentage of participating children with an anti-Rubella IgG at the cut-off level or higher (</≥ 10 IU/l) and 95%-CI by season of first vaccine (MMR-1) and age of blood sampling.

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