Human Parvovirus B19 Erythrovirus

Methods Established for Virological and Diagnostic Aspects

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Karen Marie Carlsen's Ph.D. dissertation was accepted by the Faculty of Health Sciences, University of Copenhagen, Denmark in May 2006. The public defence of this present Ph.D. dissertation took place on September 1st 2006 in Einar Lundsgaards Auditorium at The Panum Institute, Copenhagen, Denmark.

Discussion concerning epidermiology

In 1886, 1897 and 1899 erythema infectiosum epidemics were described for the first time as a clinical diagnosis with a characteristic rash becoming lattice-like with time.

In 1974, parvovirus B19 was discovered by co-incidence, and in 1983 found to be the etiology of erythema infectiosum. It is now generally accepted that parvovirus B19 epidemics occur approximately every three years in Denmark with sporadic cases occurring in between. There were no official recordings regarding parvovirus B19 epidemics in Denmark in 1991, but the fact that all pregnant women from the prospective study in 1991 (Material A) with parvovirus B19 infection were diagnosed and confirmed from August to December indicates a preceding epidemic during the first half of 1991.

The incidence of parvovirus B19 infection found to be 15.8%, (95% confidence limits 8.8–29.9%) among the 57 consecutive cases of foetal and neonatal death, may reflect an epidemic during the establishment of different methods for detection of parvovirus B19 infection in Denmark.

Fig. 1. Official opponents were: Associate Professor, Dr. Med. Hanne Colding *(second from left)*, University of Copenhagen (chairperson), Professor, Chief Physician, Dr. Med. Jørgen Serup *(right)*, Bispebjerg Hospital and Chief Physician, Dr. Med. Henrik Permin *(left)*, Bispebjerg Hospital.

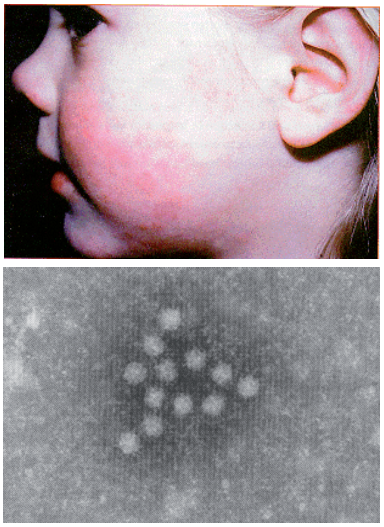

Fig. 2. EM of parvovirus B19 in serum KM1 from her own pregnancy used as positive control throughout the Ph.D. studies.

In two multicentre studies of pregnant women infected with parvovirus B19 in the second trimester, excess foetal losses of 12% were found. Parvovirus B19 infection according to Schwarz et al. accounts for approx. 2% of all second and third trimester foetal losses. Our data in study A indicates that this may be underestimated.

The incidence of parvovirus B19 infection among the 10 cases of hydrops foetalis was 40%, (95% confidence limits 13.7–78.8%) and in 44 % of the parvovirus B19 infected pregnancies a hydrops foetalis was observed. In all four cases of parvovirus B19 infection plus hydrops foetalis another possible cause of foetal death was found. In the remaining cases, parvovirus B19 infection was the only etiology found as the cause of foetal death corresponding to 4/57

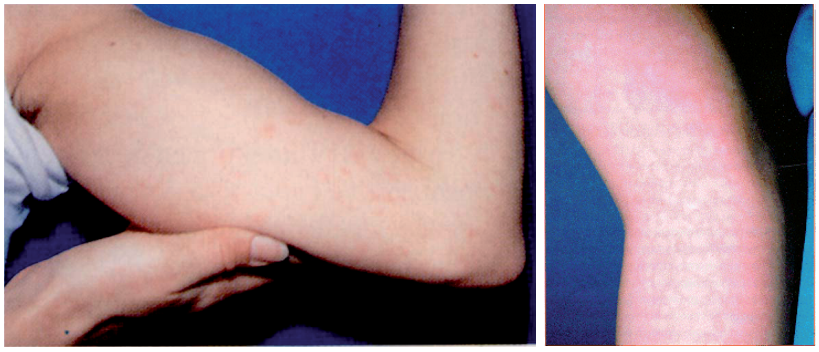

Fig. 3. Arthritis in elbow of patient with erythema infectiosum from the Department of Dermatology at Bispebjerg Hospital. Swelling and pain, causing considerable limitation of movement often occurring symmetrically, are most pronounced in fingers, hands, knees and elbows. Reactive arthritis may be with or without hydarthron and becomes lattice-like with time

(7%, confidence limits 2–17%).

A study of parvovirus B19 infection in 57 idiopathic non-immune hydrops foetalis by Jordan et al. 18%, (95% confidence limits 8.8–29.9%) contained parvovirus B19 DNA. Mark et al. found parvovirus B19 in 5/32 (16%, 95% confidence limits 5.3–32.8%) cases of hydrops. Morey et al. found that 10/37 (27%, 95% confidence limits 13.8–44.1%) non-immune foetal hydrops cases without structural malformations were parvovirus B19 positive, representing 8% of all non-immune cases of foetal hydrops.

Rates of placental transmission varying from 7% to 100% have been reported. It has been discussed whether parvovirus B19 may be teratogenic as is the case for animal parvoviruses. Following intrauterine parvovirus B19 infection most of the morbidity reported has been associated with prematurity, recovery from hydrops due to anaemia, myocarditis or residual parvovirus B19-associated haematological abnormalities. Foetal loss may occur up to 12 weeks after onset of maternal symptoms. None of the nine infected pregnant women from 1991 (Material A) reported symptoms due to the fact that they had subclinical parvovirus B19 infections. Therefore, none of the foetuses were treated for their hydrops or treated with intrauterine blood transfusion. Our findings in 1991 underline the question raised by Smoleniec et al.: whether subclinical parvovirus B19 infections during pregnancy may represent a higher risk than previously assumed. We invited all pregnant women to participate in a screening for parvovirus B19 in early pregnancy from January 1994, when the next epidemic was anticipated but surprisingly we did not find what we expected.

A retrospective study published in 2002 by Nunoue et al. between 1986 and 1999 in Japan, to clarify the relation between the time of parvovirus B19 infection in pregnancy and foetal outcome, found 13 maternal-foetal parvovirus B19 infection among 57 pairs showing serologic and/or virological evidence of parvovirus B19 infection. Nonimmune hydrops occurred at 23, 21 and 26 gestational weeks (gw) in 3 foetuses as a result of their mother's erythema infectiosum at 3, 16 and 19 (gw). Two spontaneous

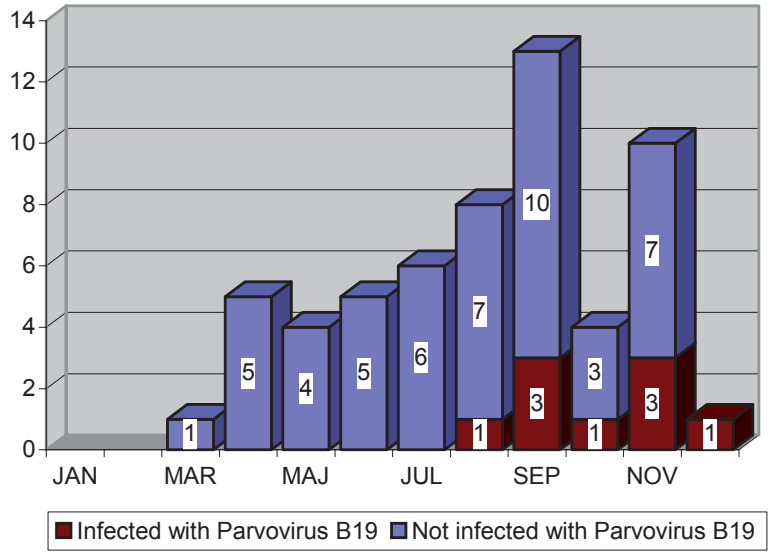

Fig. 4. Time distribution of the 57 consecutive cases of foetal and early neonatal deaths with and without parvovirus B19 infections recorded from March 15 to December 15, 1991, the National University Hospital of Copenhagen, Rigshospitalet, Denmark (Material A). All parvovirus B19 infections occurred between August and December, 1991 (P = 0.01, Fisher exact test) indicating an epidemic during the summer, autumn and early winter that year.

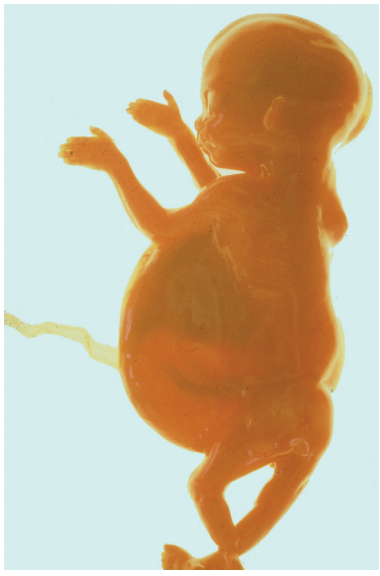

Fig. 5. Foetal Hydrops clearly visualised by transillumination. Diagnosis and intrauterine blodtransfusion is important before skin defects appear. The Department of Pathology, Rigshospitalet, The National University Hospital, Copenhagen, Denmark.

abortions after maternal infection and 8 foetuses were asymptomatic as a result of maternal infections. Parvovirus B19 DNA was detected in all 9 neonatal serum samples tested. It was concluded that maternal B19 infection throughout gestation, including the early stage after fertilization, caused foetal infection which persisted until intrauterine foetal death, with or without nonimmune hydrops, or until the neonatal period after birth.

In 1994, the first prospective consecutive screening: Parvovirus B19 infection during pregnancy took place (Material C). The parvovirus B19 IgG seroprevalence proved to be 64.6% in a population of Danish pregnant women, which is similar to the population of childbearing age in other countries. The remaining 35.4% presented no parvovirus B19 antibodies and were at risk of being infected during their pregnancy. These susceptible women were followed and tested again post partum for seroconversions. In cases of acute infection, ultrasonography was performed every 2 weeks for early detection of foetal hydrops.

The first acute parvovirus B19 infection, in the study of Material C, was diagnosed in January 1994, followed by 31 similar acute infections until the end of December, 1994. Also, 15 seroconversions were identified. A total of 46 cases of parvovirus B19 infections during pregnancy were detected at the National University hospital and Frederiksberg hospital. This indicates a second Danish epidemic during 1994 with the last case of seroconversion on June 21, 1995.

In affirmative cases of infection during pregnancy, control procedures included frequent foetal ultrasonic scanning to identify early signs of foetal hydrops or foetal anaemia due to parvovirus B19 infection, which require analysis of the foetal haemoglobin concentration in foetal blood sampled through ultrasound guided cordocentesis, but this was only to be carried out, if the foetus showed signs of stress. Relieving hydrops foetalis through aspiration of the ascites and blood transfusion, if necessary may prevent fatal complications due to transplacental spreading.

Parvovirus B19 IgM and IgG antibody tests, as well as PCR for detection of parvovirus B19 DNA, were repeated. None of the foetuses, however, developed severe hydrops foetalis. In week 22, one foetus presented transient foetal hydrops as. All of the infected women, who participated in the screening project, had a successful outcome with a healthy child apart from one 1/46 (2.2%), who had a legal abortion not related to parvovirus B19 infection; in fact the abortion was induced before results of her screening were known. All together, the outcomes of the screening project indicate that during an epidemic the rate of seroconversions is approximately 46/3,628 (1.3%) per 18 months of outbreaks. Whereas the true conversion rate is 46/1, 284+23 of the susceptible (3.5%). In total, 2/46 (4.3%) had clinical disease with erythema infectiosum, as most cases of parvovirus B19 infections are subclinical and uncomplicated. Still, complicated cases do occur sporadically. The mortality rate due to parvovirus B19 infection during the pregnancy-screening project was 0/45 (0%, 95 % confidence limits 0–7.9%).

The incidence of foetal loss among the parvovirus B19 infected pregnant women in the Danish screening project is significantly lower than the incidence 30/156 (19%, 95% confidence limits 13.5–26.3%) in a prospective study in England and Wales, but the incidence of foetal loss in the British infected group was however, similar to that of an uninfected antenatal sample, but with excess of foetal loss in the second trimester.

In order to avoid bias, we excluded parvovirus B19 patients transferred from other counties to Rigshospitalet, the National University hospital due to complicated pregnancies. However, apart from the women enrolled in the pregnancy screening, 2 women were transferred from other

counties to Rigshospitalet during the epidemic with severe foetal hydrops due to parvovirus B19. One foetus survived after aspiration of ascites under ultrasound guidance, which was performed twice in weeks 21 and 22. The second foetus with hydrops died 3 days after section at 32 weeks due to severe hydrops, in spite of aspiration of ascites, treatment with immunoglobulins and blood transfusions. The 2 umbilical cord blood samples available from the infected foetuses were found positive for parvovirus B19 IgG antibodies but negative for parvovirus B19 IgM antibodies. One of these was parvovirus B19 DNA positive. These findings indicate that foetal hydrops may exist for several weeks after a parvovirus B19 infection during pregnancy.

The State Serum Institute, Denmark, also investigated the same epidemic. A total of 10,333 serum samples were submitted to the laboratory either for serological verification of a clinical diagnosis of parvovirus B19 infection, due to exposure to parvovirus B19 or for immunological screening for a subclinical or previous infection. This selected material included samples from 286 pregnant women suspected of parvovirus infection. Investigations by Parvovirus B19 IgM and IgG IDEIATM (DAKO, Glostrup, Denmark) revealed 29/286 (10.1%, 6.7–14.0%) with parvovirus B19 IgM antibodies, i.e. a significantly higher incidence than in our material ($p<0.0001$, X2.test). Their results could point to false-positive cases due to the use of an unreliable kit, which later was removed from the market. Four of the 29 (13.8%, 3.9–31.7%) parvovirus B19 infected cases had adverse outcomes (Foetus mortuus = 2, spontaneous abortions = 2) compared to none in our material ($P = 0.02$, Fisher exact test). The epidemic period of 8 months, from February 2 to September 30, 1994, is much shorter than in our experience. The presence of parvovirus B19 IgG, in women of childbearing age ranging from 18 to 45 years old, was found to be 60%, which is a little lower than in our findings.

Discussion concerning technology

In the present studies, different methods to detect parvovirus B19 were developed and established. Evaluations of antibody tests were performed. PCR development required adjustment of several variables, such as extraction methods, $MgCl_2$ concentrations, temperatures and duration of thermal cycling. In the early phases of this investigation, attention was especially paid to pre-treatment of serum samples in order to extract parvovirus B19 DNA before the PCR. When pre-treatment consisted of heating only, described in Appendix 3 and 4, as recommended by some authors, it was difficult to obtain reproducible results. Proteinase K treatment in lysis buffer, followed by DNA extraction with saturated phenol and purified chloroform was then performed and in many aspects similar to the method used by Patou et al. From then on, there were no difficulties in obtaining reproducible results, visualized on gel electrophoresis. During the experiments with different concentrations of $MgCl_2$ in order to optimize PCR and find the necessary amount to produce the clearest bands, experiments subjecting our PCR products to either a 2% or a 1% agarose gel electrophoresis were performed. The two procedures were both satisfactory, but by using the latter method we could cut costs.

The development of parvovirus B19 antibody analysis resulted in faster and cheaper methods. We applied different antibody tests on Material A and found concordance in this small material between the different antibody kits. In conclusion, pregnant women with aborted or dead foetuses suspected of parvovirus B19 infection should be investigated for parvovirus B19 DNA, IgG and IgM antibodies in serum as well as in foetal tissue and foetal serum. Parvovirus B19, specifically IgM and IgG, were consistently found in maternal blood using either indirect immuno-fluorescence assay IFA or ELISA. Furthermore, the 8 parvovirus B19 IgM antibody positive maternal sera were all found among the nine DNA positive cases. Of the nine pregnant women with a parvovirus B19 infection four foetal serum samples were available; parvovirus B19 DNA and IgG was detected in all four, while parvovirus B19 IgM was not detectable in any of them. In order to securely diagnose an acute transplacental foetal infection, the foetal serum should be subjected to PCR with high sensitivity.

In Material A, the different methods: PCR, in situ hybridization and antibody tests were compared. Our results revealed that the first established PCR in our laboratory was superior to in situ hybridization as 9 parvovirus B19 positive foetuses were detected by PCR and confirmed by dot blot, while only 6 of these were found to be positive when tested by in situ hybridization.

In four samples subjected to PCR a positive amplification of the NS1 region could not be confirmed in the VP1 region, and in two samples a positive amplification of the VP1 region could not be confirmed in the NS1 region.

These discrepancies may be attributed to fluctuations in the sensitivity of the PCR and stress the need for amplification of more than one genomic region to avoid false-negative results. In these four cases, results were confirmed by retesting another part of the sample, which had been stored in a different Cryotube, thus excluding contamination as an explanation.

At the beginning of our studies, only first generation thermal cyclers were available and procedures much more time consuming, e.g. the new generation of thermal cyclers have improved the heating processes considerably by heating the tubes faster to a more even and precise temperature.

Liver biopsies were the most reliable source for detection of parvovirus B19 in the foetus by PCR and in situ hybridization, as earlier documented. The placenta was also a suitable source for parvovirus B19 detection by PCR, but not for detection by the less sensitive technique of in situ hybridization. These findings may be due to the massive erythropoiesis in the foetal liver. Parvovirus B19 DNA was not detected in the maternal serum samples available, possibly because the acute infection of the mother may precede the fatal affection of the foetus by 12 weeks, as discussed by Bond et al.

For a number of years, parvovirus B19 virus has been known to be an important cause of chronic anaemia in immunocompromised patients. The possible etiologic role of parvovirus B19 in other cases of anaemia was less well defined, partly due to lack of sensitivity of the diagnostic methods. Thus, a primary PCR with primer pairs designed as part of the complementary strands to the coding DNA were designed and by using a nested PCR optimized for detection of parvovirus B19 DNA, the specificity was increased and useful for the detection of low level parvovirus B19 DNA in sera from patients with chronic anaemia.

The PCR methology had a sensitivity of 50 fg B19 DNA following 30 FRcycles and 0.05 fg B19 DNA after another 18 SR cycles. In this way, a thousand-fold increase in sensitivity was obtained by adding these 18 cycles. By increasing the number of SR cycles to 24, the sensitivity was increased to 0.005 fg as detected by nested NS-region PCR corresponding to the non-structural part of the genome. This correlates fairly well with the experience gained by Patou et al., who found a sensitivity of 1 genome copy per µl of serum when a nested PCR using 35 FR cycles as well as 35 SR cycles was investigated.

Cassinotti et al. found a sensitivity that corresponded fairly well to the sensitivity described in Material B: 54% of 200 patients tested parvovirus B19 DNA positive by nested PCR and presented no parvovirus B19 IgM antibodies. Parvovirus B19 DNA can persist for several years in bone marrow cells in patients positive for parvovirus B19 IgG antibodies, who test positive for parvovirus B19 IgM antibodies in the acute phase of their disease. Corresponding to the findings in material B and as described by Török et al. in 1992, hypoplastic or aplastic crises may appear in patients with chronic anaemic conditions before parvovirus B19 antibodies can be detected. Patou et al. found that parvovirus B19 DNA detection was only possible, when nested PCR was used, in 84% of the samples. Musiani et al. found an advantage of using nested methodology, in his investigations for parvovirus B19 DNA in IgM positive serum samples from HIV-1 antibody positive patients.

From the prospective screening project (Material C), the 3,628 pregnant women were tested for parvovirus B19 IgG and IgM antibodies. Initially, using Parvovirus B19 IgG and IgM IDEIATM (DAKO, Glostrup, Denmark) which later were removed from the market and replaced by Parvovirus B19 IgG and IgM EIA, 3rd generation, Biotrin, Dublin, Ireland. During these studies some developments took place, but the comparison of 2nd generation (MKII) Parvovirus B19 IgG EIA with the 3rd generation (MKIII) kit and of the 2nd generation (MKII) Parvovirus B19 IgM EIA with the 3rd generation (MKIII) kit concludes that the analysis for parvovirus B19 IgG antibodies were reliable for the whole project: Parvovirus B19 infections during pregnancy, as well as the comparison of 2nd generation (MKII) Parvovirus B19 IgM EIA with the 3rd generation (MKIII) kit also performed on specimens from the project.

An evaluation by Schwarz et al. also concluded that Parvovirus B19 IgG and IgM EIA, 3rd generation (Biotrin,

Dublin, Ireland) were superior compared to other kits for detection of parvovirus B19 IgG and IgM antibodies. In conclusion, it was necessary, due to our in house experience, to standardize the methods for detection of antibodies, to achieve reproducible and reliable results. A combination of different methods to confirm the diagnosis was found to be of great value, in order to determine at which point of the parvovirus B19 infection the patient was diagnosed.

It was discussed at one point whether to test all the collected blood samples for the new erythrovirus V9. However, since no additional erythrovirus V9 isolates were reported when screening a large number of Danish clinical samples employing a PCR assay allowing simultaneous detection of, and distinction between parvovirus B19 and parvovirus V9, these investigations were not performed. A similar finding in a survey including Danish serum samples was performed in USA and confirmed that it was not necessary to test the serum samples in study A, B and C for erythrovirus V9 since it is not present in Danish serum samples.

Conclusions

From the early 1990s, Erythema infectiosum was no longer "just" a clinical diagnosis in Denmark, but could now be diagnosed and confirmed using the many different methods developed in the Panum Institute, and established at the Department of Clinical Microbiology at the National University Hospital, where they are still in use today, as well as in many other places. The main objective to establish reliable methods with reproducible results was fulfilled.

PCR is of great value for diagnostic purposes of parvovirus B19 infections and PCR is relevant in order to determine at which point of the infection the patient was diagnosed.

The development of antibody analysis resulted in faster and cheaper methods. Different IgM and IgG antibody tests were applied on Material A, obtained from 57 consecutive cases of foetal and early neonatal death in singleton pregnancies, recorded at the National University Hospital, Rigshospitalet, Copenhagen in Denmark, during a 9-month-period between March 15 to December 15, 1991, and found concordance in this material between the different antibody kits. In conclusion, mothers of aborted or dead foetuses suspected of parvovirus B19 infection should be investigated for parvovirus B19 DNA, IgM and IgG antibodies in serum as well as in placenta, foetal tissues and serum. For histopathological and morphological studies in situ hybridization may be used, but is too time consuming for routine analysis.

A high rate (15.8%) of foetal loss in 1991 was found due to subclinical parvovirus B19 infection during pregnancy. The causes of non-immune foetal hydrops are numerous and parvovirus B19 infection should be considered from early stages of pregnancy, when prevalent causes have been eliminated. However, parvovirus B19 should not only be considered when hydrops is present, since maternal infection throughout gestation may cause foetal infection without hydrops.

In 1994, another epidemic was anticipated; therefore, we invited all pregnant women to participate in a screening for parvovirus B19 in early pregnancy (Material C). All of the infected women had a successful outcome with a healthy child. Only 2/46 (4.3%) of the infected women presented clinical symptoms and 1/46 (2.2%) decided to have a legal abortion before the results of her screening analysis were known.

Unfortunately, a large number of pregnant women initially were diagnosed as infected due to a false-positive parvovirus IgM antibody test, therefore it is of the utmost importance that assays are tested and proved reliable, before becoming commercially available.

It was important to standardize the antibody tests, and the standard appears to be the Parvovirus B19 IgM and IgG EIA (Biotrin, Dublin, Ireland) which later received approval as "The golden standard" from the Food and Drug Association (F.D.A.) in U.S.A. in 1996 and found to be superior in Europe from 1997. The parvovirus B19 IgM enzyme immunoassay cut-off was established using receiver operating characteristic (ROC) analysis giving a sensitivity and specificity of detection of 89.1% and 99.4%, respectively. No cross-reactivity observed with rubella or other viral disease IgM that cause similar symptomologies to parvovirus B19.

The screening revealed the second diagnosed parvovirus B19 epidemic in Denmark in the period between January 1994 and the first half of 1995. Since then, parvovirus B19

epidemics have been monitored in Denmark by the State Serum Institute of Copenhagen along with other viral infections.

Future Aspects

In the present study, reliable methods were developed, and the importance of reproducible results has been described. When I finished the studies A, B and C, my wishes for the future were that my findings could provide a basis for future research, and, finally, the possibility of rapid, early diagnosis, and thus more efficient intervention or treatment of parvovirus B19 infections.

I used these methods in a study over a 15 month period from August 1996 to November 1997 for detection of parvovirus B19 in a Danish paediatric department (Appendix 9). No acute cases were detected, however, but the seroprevalence of the 226 children was determined. Again, the methods were used in a study from July 1995 to February 2000 to detect possible parvovirus B19 infections in patients with cutaneous vasculitis admitted to the dermatology department at Bispebjerg Hospital (Appendix 10). However, no sporadic cases of parvovirus B19 infections were detected.

I also wished that future procedures be focused on the possibility of screening blood products before transfusions of e.g. factor preparations and blood transfusions, which was not standard procedure, although later was considered in plasma pools. I observed with great satisfaction when investigations for parvovirus B19 erythrovirus in donors were implemented, as of January 1st 2004. In accordance with The European Pharmacopoeia, levels of parvovirus B19 DNA in plasma pools for production of anti-D immunoglobulin should not exceed 104 IU per mL. Hence, before pooling, highly viraemic donations have to be identified, and after pooling the level of parvovirus B19 DNA must be determined.

Of 503,040 donations, 29 exceeded 5×10 IU per mL of parvovirus B19 DNA (1:17, 346) proving that the parvovirus B19 DNA quantification test (LightCycler, Roche) is suitable for quantitative, routine, in-process measurement of parvovirus B19 DNA levels in plasma pools, utilizing the DNA extractor (NucliSens, bioMerieux) for nucleic acid isolation.

The cornerstone of therapy for haemophilia A and B patients is the replacement of the deficient Factor VIII and XI, respectively. Future improvement should focus on the development of safe and effective gene transfer technology.

Restriction enzyme analysis and sequencing of parvovirus B19 as a future diagnostic tool will not only identify but also evaluate the stability of genomic markers. By linking the phylogenetic tree to clinical symptoms of disease the possibility of reactivation of latent or persistent infection may be distinguished from super infections. The finding of circulating strains of parvovirus B19 in Asia reveals the importance of typing parvovirus B19 in order to recognize the spreading throughout the world. Future studies may also focus on the clinical significance and aspects of parvovirus B19 infections in subgroups i.e. chronic anaemia patients, sickle cell anaemia patients, teachers, staff in nurseries and kindergartens, hospital staff, infertile women prior to fertility treatment. Bearing in mind that 35% of women are at risk of infection with parvovirus B19 during pregnancy, a screening offer during epidemics could benefit these women.

Serious effects of most parvovirus B19 infections are rare. The clinical disease is strongly influenced by the haematological and immunologic status of the host and tends to occur mainly in immunosuppressed patients. Therefore, future studies may also be focused on further development of a vaccine, which would undoubtedly be beneficial to the above-mentioned subgroups. In the USA, a pilot study with vaccine consisting of virus capsids, without the infectious agent, was carried out on a group of anaemia patients. However, more research concerning these empty virus capsids and other vaccine suggestions needs to be carried out. The availability of an international standard serum for parvovirus B19 IgG antibodies, described in a study from 1997, allows quantitation of IgG titres in IU/ml, which will facilitate a comparison of results world wide for determining the immune response induced by the candidate parvovirus vaccines, which are currently under evaluation.

Finally, all severe cases of parvovirus B19 could be reported to a clinical database, which could serve the purpose to enlighten all aspects of parvovirus B19 infections.

Summary

The aim was to develop and establish reliable methods to detect human parvovirus B19 erythrovirus infection in Denmark. The methods were applied to the following Materials A, B and C as described here.

In Material A, consisting of 57 consecutive pregnancies, complicated by second trimester abortion, intrauterine and neonatal death, samples of placenta, maternal blood, foetal blood and liver were obtained 3 to 6 hours post abortion, delivery or neonatal death. DNA was extracted and polymerase chain reaction (PCR) amplification of fragments from two regions of the parvovirus B19 genome was carried out. In both regions, a primary and a nested PCR were performed, and the identity of the products was confirmed by Southern blot and dot blot hybridization using a digoxigenin labelled DNA probe. Parvovirus B19 DNA was also detected by in situ hybridization on sections of placenta and foetal liver. Maternal and foetal blood samples were examined for parvovirus B19 IgM and IgG antibodies. Parvovirus B19 IgM antibodies could not be detected in any of the foetal blood samples, only parvovirus B19 DNA was present. The different methods were compared and PCR was found to be superior to in situ hybridization to detect parvovirus B19 DNA. Parvovirus B19 infection was found in 9/57 (15.8%) and these 9 cases were all detected in the second half of 1991; 44.4% (4/9) of the infected cases had hydrops foetalis. The rate of parvovirus B19 infection among the foetuses with hydrops was 40.0% (4/10). None of the women presented clinical symptoms of parvovirus B19 infection, so at the time it was concluded that all pregnant women should have the opportunity of being investigated for parvovirus B19 IgM and IgG antibodies in serum, as early as possible during pregnancy. Detection of parvovirus B19 IgM would substantiate further investigations for parvovirus B19 DNA in serum and i.e. in foetal tissue and serum along with the clinical investigations. These data indicated a high rate of foetal loss due to subclinical parvovirus B19 infection during pregnancy. Therefore, a general screening for parvovirus B19 IgM and IgG in early pregnancy during the next anticipated epidemic was prepared and ready by January 1994.

In Material B, consisting of 42 patients with chronic anaemia, parvovirus B19 DNA was detected in 10, the majority of whom suffered from aplastic anaemia, haemolytic anaemia, pure red cell anaemia or myelodysplastic syndrome. Nested PCR methods with sensitivities of 0.005–0.05 fg DNA were developed. In 9 of the patients, parvovirus B19 DNA could only be detected by nested PCR as this method increased the specificity and sensitivity. Conventional PCR with a sensitivity of 50 fg parvovirus B19 DNA could only detect the DNA in one patient. In the majority of parvovirus B19 DNA positive patients, the DNA was estimated at 0.005–0.05 fg/5 µl serum.

In Material C, the prospective screening project: Parvovirus B19 infection during pregnancy, 3,628 women were tested for parvovirus B19 IgG and IgM antibodies early in pregnancy. The parvovirus B19 IgG seropositive prevalence was 64.6%, which is similar to the population of childbearing age in other countries. The remaining 35.4% had no parvovirus B19 antibodies, and were therefore susceptible to infection during their pregnancy. These women were followed and tested again post partum to detect possible seroconversions. In the event of acute infection, ultrasonography was performed every 2 weeks for early detection of foetal hydrops. Detection of foetal anaemia due to parvovirus B19 infection requires analysis of the foetal haemoglobin concentration in foetal blood sampled through ultrasound guided cordocentesis. Relieving hydrops foetalis, through aspiration of the ascites, and blood transfusion, if necessary, may prevent fatal complications due to transplacental spreading. Parvovirus B19 IgM and IgG antibodies as well as DNA testing was repeated. A total of 46/3,628 (1.3%) of the investigated corresponding to 46/1,307 (3,5%) of the susceptible were diagnosed with parvovirus B19 infection during pregnancy, 31 women tested parvovirus B19 IgM positive while 15 showed seroconversions from parvovirus B19 IgG negativity during their pregnancy. All of the infected women participating in the screening had a successful outcome with a healthy child. Only 2/46 (4.3%) had clinical symptoms, and 1/46 (2.2%) decided to have a legal abortion at 11 weeks on social indications; the result of the parvovirus B19 screening was unknown to her at the time of the abortion.

Unfortunately, a large number of pregnant women initially were diagnosed as infected due to a false-positive parvovirus IgM antibody test, therefore it is of the utmost

importance that assays are tested and proved reliable, before becoming commercially available.

In summary, the data supports the claim that the Parvovirus B19 IgG and IgM EIA (Biotrin, Dublin, Ireland) can assess the antibody status of serum from pregnant women.

The screening project revealed the second diagnosed parvovirus B19 epidemic in Denmark in the period between January 1994 and June 1995. The last pregnancy presenting seroconversion proved to be parvovirus B19 IgG negative on December 16, 1994 whereupon, a second sample proved to be parvovirus B19 IgG positive on June 21, 1995.

List of Original Papers:

The Ph.D. thesis is based on these 4 articles and 6 abstracts for posters and oral contributions:

Carlsen KM, Hornsleth A: The fifth disease - erythema infectiosum. Parvovirus infection in a family group. Ugeskrift for Læger 1990; 152: 1383–1384. Chapter XVI – Appendix 1.

Carlsen KM, Hornsleth A: Benign and life threatening Parvovirus B19 infections. Månedsskrift for Praktisk Lægegerning 1992; 2: 89–95. Chapter XVII – Appendix 2.

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Fig. 6. Benny Rosenfeld ended the disputation with a fanfare.