GESNOMA (Geneva Study group on Noma): an aetiological research on noma disease

D. Baratti-Mayer[1], B. Pittet[1], D. Montandon[1], and GESNOMA[2]

[1] *Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Geneva Hospitals, Geneva, Switzerland*

[2] *Members of GESNOMA are: Dr. D. Baratti-Mayer, Dr. I. Bolivar, Dr. J.-E. Bornand, Dr. A. Gervaix, Dr. A. Jaquinet, Dr. S. Hugonnet, Prof. A. Mombelli, Prof. D. Montandon, Prof. B. Pittet, Prof. D. Pittet, Mme M. Rusconi and Prof. J. Schrenzel*

SUMMARY

Noma is a devastating gangrenous disease that leads to severe tissue destruction in the face. It is seen almost exclusively in children living in developing countries. The exact prevalence of the disease is unknown and the cause also remains unknown. Risk factors are malnutrition, a compromised immune system, poor oral hygiene and a lesion of the gingival mucosal barrier, as well as an unidentified bacterial factor. Herpes viruses might also contribute. Studies of the buccal flora in acute phases of noma and comparison with control children are presently non-existent. Our study takes place in Niger, Africa. For each child (cases and controls), we take samples of gingival fluid, saliva, blood and mouth mucosal swabs. The samples are then analysed in Geneva in different laboratories. We control the serologies for Herpes viruses and measles. We also perform a nutritional assessment and the mucosal swabs are cultivated for the presence of viruses. The gingival flora is investigated by microarrays. These microarrays are instrumental to test for the presence of thousands of different bacteria in each clinical sample. This method allows a qualitative and quantitative description of the oral flora in children with noma and control cases.

Keywords: Noma, microarrays

1. INTRODUCTION

Noma is a gangrenous disease which leads to severe disfigurement of the face with high morbidity and mortality. Acute noma affects young children living in the poorest areas of developing countries, especially in sub-Saharan Africa. The prevalence and incidence of the disease are still unknown as is the aetiology. Risk factors for noma have been known for many years and include malnutrition, immune dysfunction, poor oral hygiene and a lesion of the mucosal gingival barrier (Baratti-Mayer et al. 2003). Due to their local immunodepressive capacity, it has also been hypothesized that *Herpesviruses* may play a role in preparing the conditions for the development of a still unknown bacterial agent with parodontal pathogenicity (Contreras et al. 1997). Previous studies suggested that *Fusobacterium necrophorum*, a parodontal pathogen, commensal of the gut of herbivores and potentially contaminating livestock, could be the bacteria implicated in noma disease (Falkler et al. 1999). Due to the regions of the globe where noma impairs and the rapidity of the evolution of the disease, microbiological studies on acute cases and case-control studies are lacking.

1.1 Material and methods

GESNOMA (Geneva Study group on Noma) is a group of healthcare workers divided into two teams, one located in Geneva and the other in Zinder, Niger. The Geneva team is multidisciplinary with the following specialities: plastic surgery, maxillo-facial surgery, dentistry, parodontology and dental hygiene, epidemiology, paediatric, infectious diseases, virology, biology and genomic. The Niger team is composed of two nurses and a driver.

The GESNOMA project started in September 2001 and is conducted in the region of Zinder, the second largest city in Niger. The children are seen in a consulting centre for noma care created by the non-governmental organization "Sentinelles" in 1992. To date, more than 600 patients affected with noma (acute cases and sequels) have attended the centre. The project is a case-control study which includes only acute cases in children less than 12 years old. Children or adults presenting with old lesions are excluded.

Each time that an acute noma case is included, our local team returns to the village of the child and includes four control children of the same age (Fig. 1). Every child (noma or control) undergoes general, facial and oral examination. We then take samples of gingival fluid (Fig. 2), saliva, blood and mucosal swabs (for the noma cases, diseased site and healthy site are sampled). In parallel, we collect epidemiological data on the child and family, nutritional habits, animal proximity, previous and recent diseases, and vaccination history. The presence of a local team is indispensable for this work to ensure adequate communication in the local languages ("haussa", "peul", "djerma") and to approach the youngest children who sometimes have never seen a "white person" previously.

Due to the presence of the local team all year long, we have had the possibility to include every new case presenting with an acute noma. The team is always the same and has been coached in sampling techniques by the same person (DBM) from the Geneva group. Each year, one of the two persons responsible for the field project travels to Niger for a periodic control and to transport to Geneva the frozen samples.

The aims of the study are:

- to describe the oral flora of acute noma cases
- to compare this flora with the one of the age-matched control children
- to control the validity of the viral theory (*Herpesviruses*)
- to control the validity of the bacterial theory (*Fusobacterium necrophorum*)
- to collect epidemiological data

Fig. 1. A member of the Geneva team with a Zinder-based male nurse at work in a village: completion of the epidemiologic questionnaire

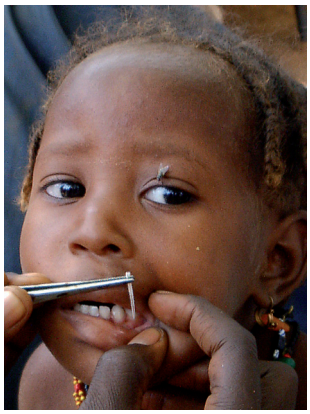

Fig. 2. Gingival fluid sampling (control child)

1.2 Laboratory analyses

All laboratory analyses are conducted in Geneva. Serologic studies consist of detection of IgG and IgM for *Herpesviruses* (*Cytomegalovirus, Epstein Barr Virus, Herpes Simplex Virus, Varicella-Zoster Virus*) and for measles virus.

A nutritional and vitamin assessment is also carried out.

A procedure was developed for virus identification in the mucosal swabs by inoculation in cell culture.

For gingival flora, DNA from plaque samples was purified and amplified with universal primers. Aliquots of PCR were pooled by sex and clinical status and cloned in seven libraries. Clones were then sequenced and compared to the public databases and 1250 valid sequences identifying different species of bacteria were obtained. These data have been used to design nuclear probes which will permit to identify the presence of thousands of different bacteria in each sample by microarray.

In parallel, we developed an original bioinformatic approach to analyse the changes in the gingival flora and to identify the predominant bacterial genes. With this technique it is possible to identify the still unrecognised bacteria and allows also a qualitative and quantitative description of the oral flora in the diseased and control children.

2. PRELIMINARY RESULTS

After an initial 9 months necessary to elaborate the protocol, obtain authorizations from the Niger government and teach the local team, we began patient inclusions in September 2001. From September 2001 to September 2006, 84 acute noma cases and 323 control children were included. The number of control children is inferior to 84 x 4 because of a certain delay in control inclusions. Our analyses are still ongoing and only partial results are available at present. For validity reasons, we will not proceed with statistical analysis until the end of the study.

The first step of our microbiological analyses shows 300 different bacterial species in the oral cavities of the children (both noma and controls). Among this flora, 40% are still unknown bacteria. *Fusobacterium necrophorum* has never been identified in any sample. Some species seem to be associated with the diseased sites and others with the control children or the healthy site alone. We are now proceeding with the second step of bacteriological analyses.

3. CONCLUSION

The plastic and reconstructive surgeons of our group have been involved in humanitarian projects for many years. Noma sequels are a surgical challenge, in particular the associated complications and functional problems such as the relapse of mouth constricture. When a child consults the noma centre of Zinder he/she receives professional oral hygiene, hypercaloric nutrition, rehydration, and antibiotic treatment. The latter is conducted in an empiric manner with the antibiotics available, but without any scientific evidence of their real efficacy. These antibiotics do not always stop the disease but, in general, they do save the child from lethal complications such as sepsis or pneumonia. All the suggested aetiological theories remain inconclusive at present. Thanks to funding by a private foundation, we were able to create this multidisciplinary group and to elaborate this research project which represents the first important aetiological project on noma disease.

ACKNOWLEDGMENTS

- Sentinelles, rte de Cery, Les cerisiers, 1008 Lausanne, Suisse
- GESNOMA-Zinder, c/o Sentinelles, B.P.27, 7000 Zinder, Niger
- Fondation Gertrude Hirzel

REFERENCES

Baratti-Mayer D, Pittet B, Montandon D, Bolivar I, Bornand J.-E, Hugonnet S, et al (2003) Noma: an "2infectious" disease of unknown aetiology. Lancet Infect Dis 3: 419–431

Contreras A, Falkler WA, Enwonwu CO, Idigbe EO, Savage KO, Afolabi MB, et al (1997) Human Herpesviridae in acute necrotizing ulcerative gingivitis in children in Nigeria. Oral Microbiol Immunol 12: 259–265

Falkler WA, Enwonwu CO, Idigbe EO (1999) Isolation of Fusobacterium necrophorum from cancrum oris (noma). Am J Trop Med Hyg 60: 150–156

Author's address: Dr. Denise Baratti-Mayer, Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Geneva Hospitals, 24 Rue Micheli-du-Crest 1211 Geneva 14, Switzerland, e-mail: dbaratti@gesnoma.org