

Long-term study on symptomless human metapneumovirus infection in hematopoietic stem cell transplant recipients

Maurizia Debiaggi¹, Filippo Canducci^{2,3}, Cristina Terulla⁴, Michela Sampaolo^{2,3}, Maria Chiara Marinozzi^{2,3}, Paolo Emilio Alessandrino⁵, Anna Amelia Colombo⁵, Daniela Caldera⁵, Letizia Zenone Bragotti⁵, Roberta Migliavacca¹, Emanuela Bianchi⁶, Egidio Romero^{1,4}, Massimo Clementi^{2,3}

¹Dipartimento Scienze Morfologiche Eidologiche e Cliniche, Sezione Microbiologia, Università di Pavia, Italy;

⁴Servizio Analisi Microbiologiche;

⁵Centro Trapianti Midollo Osseo, IRCCS San Matteo, Pavia, Italy

²Lab Microbiologia e Virologia, IRCCS;

³Università Vita-Salute San Raffaele, Milano, Italy;

⁶Azienda Ospedaliera Melegnano, Presidio Vizzolo Predabissi

SUMMARY

From October 2004 through October 2006 a study was performed to evaluate the prevalence of human Metapneumovirus (hMPV) infection in adult hematopoietic stem cell transplant (HSCT) recipients. Sequential nasopharyngeal aspirates (NPA) were collected independently from respiratory symptoms and evaluated for hMPV-RNA by polymerase chain reaction (PCR) and sequence analysis. Results indicate epidemiological and molecular differences between the 2004-2005 and 2005-2006 periods and that hMPV seems not to symptomatically affect HSCT patients or cause late respiratory sequelae. In addition, data collected suggest a hospital origin of hMPV infection in most HSCT patients during the 2004-2005 period.

KEY WORDS: Human metapneumovirus, Hematopoietic stem cell transplantation, Acute respiratory disease

Human Metapneumovirus (hMPV) is an important cause of acute respiratory tract disease in normal infants and children worldwide, and also plays a role in adults, particularly the elderly and those with comorbid conditions such as chronic obstructive pulmonary disease, asthma and cancer (van den Hoogen *et al.*, 2002; Williams *et al.*, 2004; Hamelin *et al.*, 2005; Williams, 2005; Martinello *et al.*, 2006). In adult patients with haematological malignancies and symptomatic respiratory tract infection, hMPV was recovered

with a positivity rate of 3-9% (Williams *et al.*, 2005; Englund *et al.*, 2006). Clustered cases suggesting the possibility of nosocomial epidemics in hospitalized patients were also reported (Larcher *et al.*, 2005; Honda *et al.*, 2006).

We recently described a high rate of asymptomatic and persistent hMPV infection in immunocompromised patients undergoing hematopoietic stem cell transplantation (HSCT) (Debiaggi *et al.*, 2006). From October 2004 through October 2005, we recovered positive samples from 18 out of 21 patients without differences in seasonal distribution. In addition, sequence analysis documented infection by hMPV genotype A only and were identical in the amplified region.

This previous observation led us to perform a long-term survey to evaluate the real prevalence of asymptomatic hMPV infection, and the possible appearance of late respiratory sequelae in

Corresponding author

Maurizia Debiaggi

Università di Pavia

Dipartimento di Scienze morfologiche Eidologiche e Cliniche

Sezione di Microbiologia

Via Brambilla 74, 27100 Pavia, Italy

E-mail: mdeb@unipv.it

HSCT positive recipients by collecting additional epidemiological and molecular data through two consecutive epidemic periods.

In this study we evaluated patients admitted from October 2005 through October 2006 at the Division of Hematology, IRCCS San Matteo Hospital for allogenic or autologous stem cell transplantation. At the same time, patients who experienced transplantation during the 2004-2005 period, most of whom were described in our previous survey, were included in this study for long-term monitoring. All patients were enrolled irrespective of respiratory symptoms

In the same two-year period, NPA samples from children <2 years recruited at Melegnano Hospital with acute respiratory diseases (ARDs) were evaluated as controls of hMPV seasonal prevalence. In HSCT patients, NPA samples were collected at different times points (from before HSCT to 180 days after HSCT) as described (Debiaggi *et al.*, 2006). In patients recruited from the 2004-2005 period the presence of hMPV RNA in NPA samples was evaluated from 180 days up to 14 months after transplant.

In all patients clinical data were collected and examined for sign or symptoms of respiratory tract infection or late respiratory sequelae.

Detection of hMPV RNA in NPA samples was performed by reverse transcription (RT)-polymerase chain reaction (PCR) as described (Debiaggi *et al.*, 2006). Briefly, viral RNA was extracted from samples using a Qiagen RNA minikit (Qiagen), reverse transcribed by use of random hexamers and amplified by use of a primer mix including 1 forward and 2 reverse primers to amplify a 150-bp region of the N gene of all known or possible hMPV subtypes.

Amplified PCR products were separated by agarose gel electrophoresis and sequenced directly by use of BigDye Terminator v3.1 and a ABI 3100 sequencer (Applied Biosystems). Alignments of sequences and phylogenetic relationships were evaluated as previously reported in detail (Debiaggi *et al.*, 2006).

From October 2004 through October 2006, 53 adult HSCT patients (23 in 2004-2005 and 30 in 2005-2006 period) recruited at the Division of Hematology, IRCCS san Matteo Hospital were evaluated. In the first year, twenty-one out of the 23 were allogenic HSCT patients and belonged to the group of patients included in our previ-

ous study, and the remaining two were autologous HSCT.

From patients recruited in 2004 and 2005, a total of 168 NPA samples (125 obtained in the 2004-2005 and 43 in the 2005-2006 two years follow-up) were examined. The presence of hMPV RNA sequences was detected in 58 (34.5%) out of 168 NPA samples collected from 19 out of 23 enrolled patients (82.6%, 18 allogenic and 1 autologous). Fifty-five (94.8%) out of the 58 positive samples were collected in the 2004-2005 period. From the 30 patients enrolled in the 2005-2006 period (10 allogenic and 20 autologous HSCT) 133 NPA samples were obtained and evaluated for presence of hMPV RNA. Viral RNA sequences were detected in 3 (2.2%) out of 133 samples from 2 (6.6%, one allogenic and one autologous) out of 30 patients.

In children with ARDs, hMPV RNA was detected in 37 out of 244 (15.1%) in the 2004-2005 and in 30 out of 132 (23%) in the 2005-2006 period. Monthly distribution of positive samples from October 2004 to October 2006 in both HSCT and pediatric patients is reported in Figure 1. In children a similar prevalence was observed throughout the two seasonal periods. Positive samples were detected mainly in winter and spring with incidence peaking in January-February in 2004-2005 and in March-April in 2005-2006 (Figure 1a). A different pattern in hMPV distribution and prevalence of positive samples was observed in HSCT patients between the two years with high prevalence isolation throughout the entire 2004-2005 seasonal period and very low prevalence only in February-March throughout the following year. Sequencing studies revealed hMPV genotype A in all specimens from HSCT patients, nevertheless nucleotide comparison of amplified sequence from samples collected in the 2004-2005 period showed a 100% similarity. Three specific mutations (2.5% difference) were instead observed in isolates amplified in the 2005-2006 season.

No specific respiratory symptoms were documented in HSCT patients at the time of hMPV detection in NPA samples and only mild upper respiratory tract symptoms (rhinorrhea or dry cough) were present in 9 out of 19 positive patients in the first period and in one out of two positive patients in the following season. In addition, long-term monitoring of patients recruited in 2004-2005 period, when most of them were

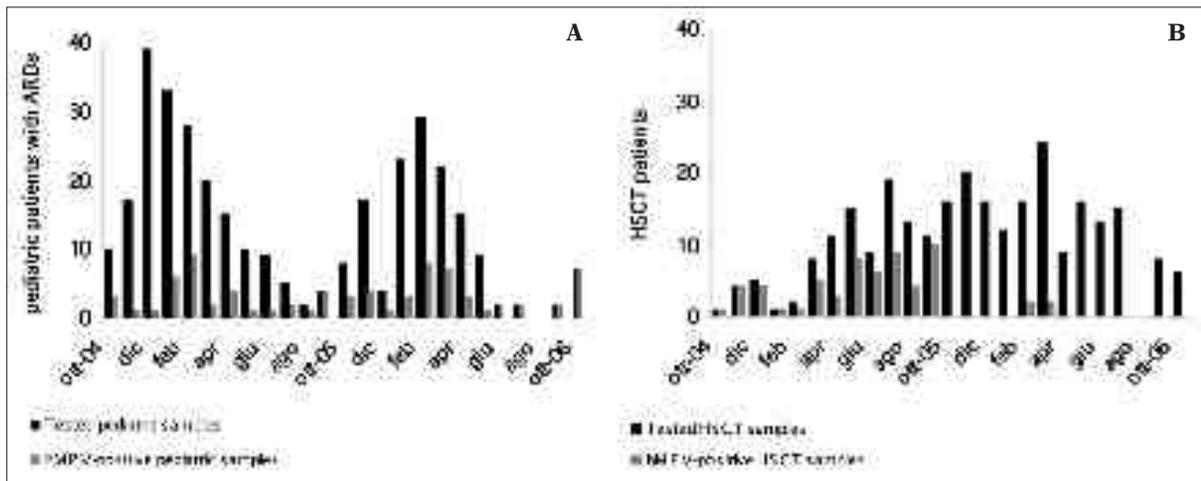


FIGURE 1 - Human metapneumovirus (hMPV) infection in pediatric patients with acute respiratory diseases (1a) and in HSCT recipients (1b) from October 2004 to October 2006. hMPV positive samples (striped bars) and total number of tested samples (black bars) per month are shown.

persistently infected, showed no late respiratory sequelae.

In this long-term prospective study we documented epidemiologic and molecular differences in hMPV infection both in two groups of patients (HSCT and pediatric) and during two epidemic periods in transplanted patients. We evaluated 23 HSCT patients, including 21 who had an allogenic and two who underwent an autologous transplantation in the 2004-2005 period and 30 patients including 10 allogenic and 20 autologous in the 2005-2006 period. Though we compared groups having a different distribution between autologous and allogenic transplants during the two epidemic periods, our results about the observed difference in prevalence would not change even considering only the allogenic HSCT. These patients are comparable in number during the two periods and are the most numerous in the study.

The hMPV seasonal distribution as well as the prevalence in the two consecutive periods, when referred to the pediatric patients, is similar to that obtained in several studies and reflects the effective yearly virus circulation (Maggi *et al.*, 2003, Kahn, 2006). In HSCT patients we observed a higher prevalence and a lack of seasonal peaks during the 2004-2005 period compared to the following period in the same patient group and in pediatric patients. These results are consistent with an intrahospital viral spread in a large proportion of these transplanted patients. The molecular

results obtained in this study support such virus circulation, since possibly the same viral strain was detected during the 2004-2005 period, and two distinct viral strains were identified in the 2005-2006 period. On the other hand, viral spread in hospitalized patients has also been reported in recent papers (Larcher *et al.*, 2005, Honda *et al.*, 2006). This survey revealed that hMPV infection in HSCT patients observed in this study is always asymptomatic and patients suffer no late respiratory sequelae. Nevertheless other studies addressing symptomatic patients found hMPV infection in about 9% of adults with hematologic malignancies (Williams *et al.*, 2005, Englund *et al.*, 2006), so that symptomatic infection cannot be excluded and the pathogenic (or co-pathogenic) role of the virus in these patients remains unclear.

In conclusion our data indicate that hMPV may be transmitted among hospitalized immunocompromised patients. Furthermore in HSCT patients hMPV infection is frequently asymptomatic suggesting that further research is needed to evaluate the virus-host interactions and the role of the immune system both on protection and eventually in the pathogenic mechanism of the disease caused by hMPV.

ACNOWLEDGMENTS

This research was founded by the IRCCS Policlinico San Matteo (grant 08058605) and Università di Pavia, Fondo Ateneo di Ricerca 2006.

REFERENCES

- DEBIAGGI, M., CANDUCCI, F., SAMPAOLO, M., MARINOZZI, M.C., PAREA, M., TERULLA, C., COLOMBO, A.A., ALESSANDRINO, P.E., ZENONE BRAGOTTI, L., ARGHITTU, M., GOGLIO, A., MIGLIAVACCA, R., ROMERO, E., AND CLEMENTI, M. (2006). Persistent symptomless human Metapneumovirus infection in hematopoietic stem cell transplant recipients. *J. Infect. Dis.* **194**, 474-478.
- ENGLUND, J.A., BOECKH, M., KUYPERS, J., NICHOLS, W.G., HACKMAN, R.C., MORROW R.A., FREDRICKS, D.N., AND COREY, L. (2006). Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. *Ann. Intern. Med.* **144**, 344-349.
- HAMELIN, M.E., COTE, S., LAFORGE, J., LAMPRON, N., BOURBEAU, J., WEISS, K., GILCA, R., DESERRES, G. AND BOIVIN, G. (2005). Human Metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. *Clin. Infect. Dis.* **41**, 498-502.
- HONDA, H., IWAHASHI, J., KASHIWAGI, T., IMAMURA, Y., HAMADA, N., ANRAKU, T., UEDA, S., KANDA, T., TAKAHASHI, T., AND MORIMOTO, S. (2006). Outbreak of human Metapneumovirus infection in elderly inpatients in Japan. *J. Am. Ger. Soc.* **54**, 177-180.
- LARCHER, C., GELTNER, C., FISHER, H., NACHBAUR, D., MULLER, L.C., AND HUEMER, H.P. (2005). Human Metapneumovirus infection in lung transplant recipients: clinical presentation and epidemiology. *J Heart Lung Transplant.* **24**, 1981-1901.
- MAGGI, F., PIFFERI, M., VATTERONI, M., FORNAI, C., TEMPESTINI, E., ANZILLOTTI, S., LANINI, L., ANDREOLI, E., RAGAZZO, V., PISTELLO, M., SPECTER, S., AND BENDINELLI, M. (2003). Human Metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. *J. Clin. Microbiol.* **41**, 2987-2991.
- MARTINELLO, R.A., ESPER, F., WEIBEL, C., FERGUSON, D., LANDRY, M.L., AND KAHN, J.S. (2006). Human Metapneumovirus and exacerbations of chronic obstructive pulmonary disease. *J. Infect.* **53**, 248-254.
- KAHN, J.S. (2006). Epidemiology of human Metapneumovirus. *Clin. Microbiol. Rev.* **19**, 546-557.
- VAN DEN HOOGEN, B., DE JONG J.C., GROEN, J., KUIKEN, T., DE GROOT R., FOUCHIER, R.A.M. AND OSTERHAUS A.D.M.E. A newly discovered human Metapneumovirus isolated from young children with respiratory tract disease. *Nat. Med.* **7**, 719-724.
- WILLIAMS, J.V., HARRIS, P.A., TOLLEFSON, S.J., HALBURNT-RUSHHH, L.L., PINGSTERHAUS, J.M., EDWARDS, K.M., WRIGHT, P.F., AND CROWE, J.E. JR. (2004). Human Metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N. Engl. J. Med.* **29**, 443-450.
- WILLIAMS, J.V. Human Metapneumovirus: an important cause of respiratory disease in children and adults. *Curr. Infect. Dis. Resp.* **7**, 204-210.
- WILLIAMS, J.V., MARTINO, R., RABELLA, N., OTEGUI, M., PARODY, R., HECK, J.M., AND CROWE, J.E. JR. (2005). A prospective study comparing human Metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. *J. Infect. Dis.* **192**, 1061-1065.