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Detection of parvovirus B19 in the lower respiratory tract

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ABSTRACT

Background: Human parvovirus B19 infection generally displays a self-limiting course followed by viral clearance; although, in some cases, persistent infection may occur. Few cases of severe pulmonary disease following primary infection in both immunocompetent and immunocompromised patients were reported.
Objectives: To investigate the prevalence and clinical impact of parvovirus B19 in the lower respiratory tract.
Study design: The prevalence of parvovirus B19-DNA was evaluated by Real-Time PCR in 264 bronchoalveolar lavages (BAL) from 189 adult patients over a full-year period and related to demographic characteristics, underlying pathologies, immune status, admission to intensive care unit, mortality within 28 days, and discharge diagnosis.
Results: Parvovirus B19-DNA was detected in 7/189 (3.7%) patients, without significant association to demographic characteristics, immune status, transplant versus non-transplant status, admission to intensive care unit, presence of haematological conditions. In two lung transplant recipients surveillance specimens were positive to B19. Four of the remaining five patients presented respiratory insufficiency. A significant association to mortality was found, as 3/7 (42.9%) positive patients died within 28 days. No patient presented serological evidence of recent or acute infection and viremia.
Conclusions: Parvovirus B19 may be detected at low frequency in BAL specimens from patients with different pathological backgrounds. This finding could be due to chronic infection with virus persistence in the lower respiratory tract, also in the absence of symptoms unequivocally attributable to B19. The high rate of mortality warrants the need for further studies to evaluate the opportunity to consider parvovirus B19 in the diagnostic work-up of lower respiratory tract infections.

1. Background

Human parvovirus B19 is a worldwide distributed single-stranded DNA virus usually infecting rapidly dividing cell lines, such as bone marrow erythroid progenitor cells.[1] Most persons are infected by 15 years of age and about 80% of the population is seropositive by the age of 50 years. Infection is most common in late winter or early spring[2] and occurs through exposure to infected respiratory droplets or blood products or vertically from mother to fetus. Most persons with B19 infection are asymptomatic or present mild, aspecific, cold-like symptoms. Other clinical conditions associated to parvovirus B19 infection include erythema infectiosum, arthropathy, and hydrops fetalis in immunocompetent individuals; haematologic diseases, including aplastic crisis, chronic red cell aplasia, and idiopathic thrombocytopenic purpura, in immunocompromised patients. A link to parvovirus B19 infection has been hypothesized for various skin eruptions, neurologic diseases, rheumatologic diseases, vasculitic and myocarditic syndromes, and autoimmune hepatitis.[3,4] Few cases of severe pulmonary disease in both immunocompetent and immunocompromised patients have been reported in literature[5–13]; however, at our knowledge, no study has systematically investigated the presence of parvovirus B19 in lower respiratory tract specimens.

2. Objectives

The aim of this study was to evaluate the prevalence and clinical impact of parvovirus B19 in bronchoalveolar lavage (BAL) specimens from hospitalized adult patients.

Abbreviations: BAL, bronchoalveolar lavage; GEq, genome equivalents.

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3. Study design

All the 264 consecutive BAL samples collected over a period of 12 months at the Virology Unit of the Azienda Ospedaliero-Universitaria San Giovanni Battista in Turin and obtained from 189 patients (men/women, 121/78; mean age ± standard deviation, 57.8 ± 15.9 years; range, 16–84) were studied. Multiple BAL procedures (mean, 3.2; range, 2–6) were performed on 30 patients. Samples from the same patient that were collected less than 2 weeks apart were excluded; each sample and episode was evaluated independently. Bronchoalveolar lavage procedure was performed for investigating the cause of unexplained fever and/or respiratory symtpoms (including dyspnea, cough, haemoptysis) and/or new infiltrates on chest X-ray or in the absence of the above-mentioned conditions for cheking up a previous positivity or as routine follow-up in lung transplant recipients at month 1 post-transplantation and subsequently at 3-month intervals. In lung transplant recipients histopathological evaluation (periodic acid-Schiff, Masson's trichrome, and haematoxylin and eosin stains) on transbronchial lung biopsy was routinely performed. The BAL procedure was carried out as previously described[14] and samples were thawed and liquefied with 1:1 N-acetylcisteine. Based on the clinical chart review, the following features were recorded: demographic characteristics; microbiological/virological (including a molecular panel able to detect 16 respiratory viruses [i.e. human cytomegalovirus, human herpesvirus-6 and -7, Epstein–Barr virus, herpes simplex virus, human enteroviruses, human rhinoviruses, human bocavirus, human coronaviruses, influenza A and B viruses, parainfluenza viruses 1–3, respiratory syncytial virus, adenoviruses], as previously described)[15] results on BAL specimen; underlying pathologies: in particular, transplantation, haematological conditions, immune status (immunosuppression due to anti-rejection treatment in transplant recipients, chemotherapy, HIV infection or long-term use of corticosteroids); admission to intensive care unit; mortality within 28 days from admission and/or sampling; year period of BAL sampling; and discharge diagnosis (made according to the International Classification of Diseases—Italian version 2002, based on the 9th revision Clinical Modification [ICD9CM], codes 480.XX–486.XX for pneumonia; 460.XX–466.XX for acute respiratory insufficiency or other acute respiratory conditions; 490.XX–519.XX for other pneumopathies; 491.21 for acute exacerbations of chronic obstructive pulmonary disease; 996.8 for transplant complications; 33.22 for bronchoscopy).

Nucleid acid extraction was performed using the automated NucliSens easyMAG platform system (bioMeriéux, Marcy l'Etoile, France), according to the manufacturer's instruction. Real-Time PCR assays were performed with the 7300 Real-Time PCR System (Applied Biosystems, Monza, Italy), by using TaqMan platform. Primers for parvovirus B19-DNA detection were designed within the VP1 and VP2 genes (accession number AY768535; primer F 5′-ACATGCACACCTATTTTCCCAATAA-3′, primer R 5′-TGTTCCATACAGAACCCACCATT-3′; probe (TAMRA) 5′-AGCAATATACAGATCAAATTGAGCGCCCCC-3′) using the Primer Express 3.0 Software (Applied Biosystems). The assay was carried out in 25 μl volume containing 5 μl of extracted specimen or plasmid dilution, 1× Master Mix (Platinum qPCR supermix—UDG with ROX, Invitrogen), 250 nM of each primer, 125 nM of probe. Uracil-DNA glycosylase was used to eliminate PCR 'carry over' contaminations from previous PCR reactions.[16] The PCR cycling protocol was as follows: 50 °C for 2 min, initial denaturation step at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing and extension). Standard curves for the quantification of DNA were constructing by plotting the threshold cycle against the logarithm of serial 10-fold dilutions of the corresponding plasmid. Amplifications

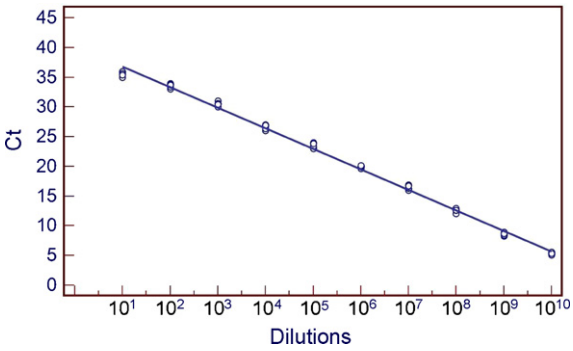

Fig. 1. Dynamic range of Real-Time PCR for parvovirus B19. Ct, threshold cycle; regression line and 95% confidence intervals for dilutions from 10^1 to 10^{10} copies/reaction are reported.

data were analyzed by the Sequence Detection System Software (Applied Biosystem). Specimens were subjected to simultaneous TaqMan PCR detection of the housekeeping gene human Glycerin-aldehyde-3-phosphate-dehydrogenase (GAPDH) as internal control, as previously described.[17] Results were considered acceptable only in the presence of GAPDH with a threshold cycle (Ct) lower than 39. The assay had a detection limit of 10 copies/reaction, thus giving, based on the analytic procedure, a value of 125 genome equivalents (GEq)/ml of BAL. Dynamic range of Real-Time PCR for parvovirus B19 is shown in Fig. 1. A "nucleotide–nucleotide blast" search for short nucleotide sequences performed at the National Centre of Biotechnology Information and the National Library of Medicine web site (available at: http://www.ncbi.nlm.nih.gov) confirmed that the primers used should not amplify other viruses pathogenic to humans. As a confirmatory test, a nested PCR that amplifies the VP1 region of parvovirus B19 was also performed, as previously described.[18]

Serology for parvovirus B19 was obtained from clinical charts or, in the available serum samples, was measured by commercial enzyme immunoassay (NovaLisa parvovirus B19 IgM and IgG, NovaTec Immundiagnostica, Dietzenbach, Germany).

For descriptive statistics, raw data, percentages, mean and median were used. For statistical analysis, chi square test and Student's *t*-test were used, as appropriate. A p-value < 0.05 was considered statistically significant.

4. Results

Overall, parvovirus B19-DNA was detected in 7/264 (2.6%) specimens from 7/189 (3.7%) patients by Real-Time PCR assay and confirmed by the nested PCR. Main features of patients with and without parvovirus B19 in BAL samples are reported in Table 1. The prevalence of parvovirus B19 positivity did not significantly differ in relation to demographic characteristics, immune status, transplant versus non-transplant status, admission to intensive care unit, presence of haematological conditions. In the two lung transplant recipients positive to B19 in surveillance BAL specimens, histopathological evaluation disclosed no finding suggestive of viral infection. Parvovirus B19 was significantly associated to mortality within 28 days, as 3/7 (42.9%) positive patients required mechanical ventilation for respiratory insufficiency and died within 28 days. As regards seasonality, all the specimens positive to parvovirus B19 were collected in autumn or winter. Overall, serology for parvovirus B19 was available for 106 patients: 77 (72.6%, including six of the seven positive to parvovirus B19-DNA on BAL specimen) with evidence of past infection (IgG positive, IgM negative) and none with evidence of acute or recent infection. Parvovirus B19 load ranged from 125 to 12943 GEq/ml BAL (median, 125) and did not significantly differ in relation to the above-mentioned patient

Table 1
Main features of patients with and without parvovirus B19 in bronchoalveolar lavage specimens.

Characteristics	Total	Parvovirus B19		
	N (%)	*N*+ (%)	N− (%)	*P*-value
Patient				
Total	189	7 (3.7%)	182 (96.25%)	–
Male	121 (64.0%)	4 (57.1%)	117 (64.3%)	NS
Female	68 (36.0%)	3 (42.9%)	65 (35.7%)	
Mean age	57.8 ± 15.9 (16–84)	62.0 ± 14.9 (38–78)	57.8 ± 16.2 (16–84)	NS
Immune status				
Competent	65 (34.4%)	3 (42.9%)	62 (34.1%)	NS
Compromised	124 (65.6%)	4 (57.1%)	120 (65.9%)	
Transplant	63	2 (28.6.2%)	61 (33.6%)	
SOT	54	2 (3.7%; lung)	52 (96.3%)	NS
BMT	9	0	9 (100%)	
Non-transplant	126	5 (71.4%)	121 (66.4%)	
Admission to ICU	24 (15.0%)	3 (12.5%)	21 (87.5%)	NS
Mortality within 28 days	21 (13.1%)	3 (14.3%)	18 (85.7%)	<0.05
Haematological condition	40 (21.2%)	2 (5.0%)	38 (95.0%)	NS
Seasonality	264 specimens			
Spring + summer	81	0	81 (100%)	NS
Autumn + winter	183	7 (4.1%)	139 (95.9%)	

SOT, solid organ transplant; BMT, bone marrow transplant; ICU, intensive care unit.

Table 2
Main features of patients positive to parvovirus B19-DNA in bronchoalveolar lavage.

Sex/age	Underlying condition	Immune status	Clinical diagnosis	Mortality	Viral load (GEq/ml)	Other pathogens
F/59	Haematological malignancy on chemotherapy	Compromised	Respiratory insufficiency	No	705	Human herpesvirus-6, human rhinovirus
M/59	Haematological malignancy on chemotherapy	Compromised	Pneumonia, sepsis	Yes (at 3 days)	<125	Epstein–Barr virus, bacteria
F/78	Acute respiratory condition	Competent	Respiratory insufficiency	Yes (at 26 days)	<125	No
M/38	Lung transplantation	Compromised	Follow-up bronchoscopy	No	370	Human cytomegalovirus
M/60	Acute respiratory condition	Competent	Respiratory insufficiency	Yes (at 1 day)	<125	Human rhinovirus
M/78	Acute respiratory condition	Competent	Respiratory insufficiency	No	<125	Human herpesvirus-7, human rhinovirus
F/43	Lung transplantation	Compromised	Follow-up bronchoscopy	No	12943	Human cytomegalovirus, human herpesvirus-7

GEq, genome equivalents.

characteristics. Main features of parvovirus B19-positive patients are reported in Table 2. Real-Time PCR assay for parvovirus B19 resulted negative in all the available six serum specimens from the patients positive on BAL samples.

5. Discussion

Parvovirus B19 is a common cause of infection worldwide and definitive diagnosis is made by the detection of IgM or viral DNA in serum samples, as well as B19-DNA may be detectable in respiratory secretions. Generally, B19 infection of immunocompetent individuals displays a self-limiting course followed by clearance of the virus from the host; although, in some cases, persistent infection may occur despite the presence of IgG antibodies. Beside blood and bone marrow, the persistence of parvovirus B19-DNA has been shown in skin, synovial, tonsils, liver, myocardial tissue, and kidney.[19–25] Although parvovirus B19 infection and persistence have been associated with a wide range of diseases, the finding of viral DNA in different tissue specimens argued against its possible pathogenic role in some clinical contexts,[22,24] suggesting that the detection of B19-DNA is not sufficient to postulate a relationship between the virus and specific diseases. In the present study we investigated the presence and clinical impact of parvovirus B19 in BAL specimens. Few reports on parvovirus B19-associated pneumonia in immunocompromised and immunocompetent patients as a result of primary infection have been described in literature, while no study has investigated the presence of parvovirus B19-DNA in lower respiratory tract specimens from a large group of hospitalized adult patients. Based on our results, parvovirus B19-DNA was found in a small percentage of patients with different pathological backgrounds, without evidencing any significant prevalence in a specific subgroup of patients. As regards the potential relationship with a particular clinical pattern, although five of the seven positive patients presented an acute respiratory condition, in the two lung transplant recipients B19-DNA was found also in surveillance BAL. This seems to argue against a possible pathogenic role of parvovirus B19 and the virus could represent an innocent bystander, the replication of which has been favoured by the impaired pulmonary background. The chronicity of parvovirus B19 infection in cryptic sites, in the absence of viremia, because of delayed (or no) clearance from these areas has been suggested for the cerebrospinal fluid. This has been evidenced because cerebrospinal fluid is routinely taken as part of routine investigation in new cases of leukemia, the pathogenesis of which has been hypothesized to be related to parvovirus B19, and is supported by the fact that B19 is known to cause encephalitis.[26] Similarly, based on our results, it is likely that another cryptic site of viral persistence is represented by the lower respiratory tract. Moreover, it is to note that viral load was moderate-low in both symptomatic and surveillance cases and that all the specimens, but one, presented at least a coinfection

with another virus, mainly herpesviruses or rhinovirus. However, it may be likely that also herpesvirus DNA finding is attributable to latency in lung, as previously described.[17] On the other hand, the presence of respiratory insufficiency in four of seven patients with B19 positivity in BAL specimen and frequent association with mortality within 28 days (42.9% of the cases) suggests the need for further investigation of the presence of parvovirus B19 in BAL and lung biopsy specimens and of its role in different clinical settings, such as intensive care unit.

In conclusion, our results suggest that parvovirus B19-DNA may be detected at low frequency in BAL specimens obtained from hospitalized adult patients with different pathological backgrounds. This finding could be due to chronic infection with virus persistence in the lower respiratory tract, also in the absence of symptoms unequivocally attributable to B19; nevertheless, the high rate of mortality in the few positive cases warrant the need for further studies to evaluate the opportunity to consider parvovirus B19 in the diagnostic work-up of lower respiratory tract infections.

Conflict of interest

None.

Acknowledgement

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