

***Mycobacterium genavense* in AIDS patients, report of 24 cases in Italy and review of the literature**

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Abstract. *Mycobacterium genavense* is a frequently missed agent of disseminated disease in AIDS patients. The increasing frequency with which such organism is being isolated in Italy suggested a comparison of local survey with data reported in literature. Isolates presumed to belong to the species *M. genavense* were centralized and identified by means of genomic sequencing and/or HPLC analysis of cell wall mycolic acids; clinical data were obtained from relevant patients' record and collected using a proper questionnaire. In 24 cases in which this organism has been isolated in Italy *M. genavense* was grown, prevalently

from blood, in liquid medium after an average of six weeks of incubation. In overwhelming majority, patients were males, presented other opportunistic diseases and were characterized by very low CD₄⁺ counts (average 23/μl); most frequent symptoms were fever, anemia and weight loss. All but two patients, who died before the mycobacterial infection was diagnosed, were treated with at least three drugs; the mean survival was close to one year. A review of literature reports revealed a wide overlapping of clinical and microbiological features.

Key words: AIDS, Disseminated infection, *Mycobacterium genavense*

Introduction

Mycobacterial infections are a major complication of severely immunocompromised HIV-positive patients. Besides *Mycobacterium avium*, whose role, recognized since the beginning of the AIDS epidemic [1], is well known, a number of other nontuberculous mycobacteria have been found responsible of disseminated infections which heavily impair expectancy and quality of life of HIV-infected patients [2]. Among these, *Mycobacterium genavense* represents probably much more than a simple curiosity as it is still considered. Its cultural features make in fact this organism 'invisible' to laboratories whose search of mycobacteria relies only on solid media and it is, in many cases, missed also by experienced centers which implemented liquid cultures.

Although the first detection [3] of a disseminated infection due to *M. genavense* in Italy is practically

contemporary, in 1992, with the recognition of this novel organism [4], it is only from 1994 that its isolation ceased to be considered as an exceptional event; in three years, in fact, 22 strains of *M. genavense* were isolated in seven different laboratories.

Microbiological and clinical features of 24 cases of infection due to *M. genavense* in AIDS patients are described in this paper and case reports from the literature are reviewed.

Material and methods

M. genavense was suspected whenever acid fast bacilli found in liquid medium failed to grow on conventional solid media. Isolates presenting such features found in various Italian laboratories were centralized in Florence for further characterization. For presumptive screening of *M. genavense* strains, with aliquots of

original liquid media were seeded in parallel: two vials of radiometric broth (Bactec Becton Dickinson, USA) presenting pH 7 and pH 6.2, respectively, and a plate of Middlebrook 7H11 medium enriched with 0.2% mycobactine J (Rhône-Mérieux, France) [5]; from a suitably grown subculture in pH 6.2 radiometric broth the NAP test (Becton Dickinson) was performed according manufacturer's recommendations [6].

Isolates characterized by a more rapid growth kinetics in acid, than in neutral, broth [3, 7] and by inhibition by NAP [3] were definitively speciated resorting to HPLC analysis of cell wall mycolic acids and to partial 16S rRNA gene sequencing. HPLC was performed, as reported previously [8], on colonies grown on mycobactine-enriched medium; in two cases in which the rapid loss of vitality of the strains did not allow the

growth of colonies the analysis was attempted directly on the centrifuged pellet of broth cultures. 16S rRNA sequencing was performed on PCR-amplified nucleic acids extracted from liquid cultures [9]. Patients data were collected using a proper questionnaire by reviewing relevant clinical records.

Results

Twenty-four strains passed through the above mentioned screening and all of them were subsequently assigned by further investigations to the species *M. genavense*. In none of the 13 cases in which both genetic sequencing and HPLC analysis were performed any disagreement was detected, genetic and lipidic analysis

Table 1. Patients characteristics and microbiological features of Italian cases of *M. genavense* disseminated infection

Years of diagnosis	Sex ^a	Age	Risk factor	Isolation sites	Number of isolations	Samples in which AFB were seen	CD ₄ ⁺ count per µl	Number of drugs used	Survival (months)
1992	M	34	Homosexual	Blood	1	Stools	27	3	0
1993	M	47	Homosexual	Blood	1	Stools, duodenal biopsy	16	3	12
1994	M	32	Drug addict	Bone marrow	1	Bone marrow	10	4	1
1994	M	30	Drug addict	Blood	1	Stools	65	3	13
1994	M	31	Drug addict	Lymph node	1	Lymph node, stools, duodenal biopsy	10	4	19
1994	M	27	Homosexual	Blood	1		20	3	– ^b
1995	F	31	Heterosexual	Blood, stools, bone marrow	7	Stools	49	3	> 26 ^c
1995	M	26	Drug addict	Blood, stools	10	Stools	18	4	12
1995	M	36	Drug addict	Blood	2		16	3	19
1995	M	29	Homosexual	Blood	8	Stools	37	3	5
1995	M	31	Drug addict	Blood	2		1	0	0
1995	M	31	Drug addict	Blood	7	Stools, lymph node	14	4	3
1995	M	36	Drug addict	Sputum	1	Sputum, stools	24	3	8
1996	M	46	Homosexual	Blood	5	Blood, stools	44	3	> 8 ^c
1996	M	34	Drug addict	Blood	4	Sputum, stools, gastric biopsy	2	3	1
1996	F	32	Heterosexual	Spleen	1	Spleen	26	4	> 17 ^c
1996	M	33	Drug addict	Blood	1		55	0	0
1996	M	48	Heterosexual	Blood	5	Stools, sputum, duodenal biopsy	1	3	6
1996	M	33	Homosexual	Stools	2	Stools, gastric biopsy	9	3	11
1996	F	26	Heterosexual	Blood, stools	6	Blood, stools	19	3	> 22 ^c
1996	M	35	Drug addict	Bone marrow	2	Bone marrow	20	4	> 19 ^c
1996	M	33	Drug addict	Blood	1	Duodenal biopsy	9	3	> 10 ^c
1996	M	32	Homosexual	Blood	3	Bone marrow	50	3	14 ^b
1996	M	41	Drug addict	Blood	12	Stools, sputum, bone marrow	14	4	> 20 ^c

^a M, male; F, female.

^b Lost at follow-up.

^c Still living.

es were performed alone on three and eight isolates respectively.

Isolates came from seven different laboratories, three of which (5 cases each one), accounting for over 62% of our survey. The distribution of the isolates in the years reveals a constant increase with the time (Table 1).

Among the isolating laboratories only one did not use radiometric system for cultures of mycobacteria; in this case the growth was achieved in the broth of biphasic system Septi-Chek AFB (Becton Dickinson) periodically monitored for presence of acid-fast organisms by means of blind smears. The average time required to detect positive cultures was 43.8 days.

Wide variability characterized *M. genavense* strains, beside a majority presenting very slow growth kinetics, weak vitality, and mycobactine-dependence, a few isolates grew within two weeks and, two of them, also on unenriched Middlebrook 7H11 medium; several strains survived also standard N-acetyl-L-cysteine-NaOH decontamination as shown by isolations from stools and sputum.

The source of strains (Table 1) provided evidence of disseminated infection in 20 (83%) of the cases characterized by isolations from blood and/or bone marrow. Other clinically significant sources include spleen and a cervical lymph node; only in one case the isolation from sputum did not allow to ascertain the clinical relevance of the finding. Four patients with disseminated infection grew *M. genavense* also from stools.

Ten patients yielded *M. genavense* isolation only once, in the others the isolations ranged from two (in 4 cases) to 12 with an overall average of 3.54.

Pathologic specimens, often originating from body districts different from those which grew *M. genavense* in culture, revealed acid fast bacilli in the majority of patients (83%); in 15 of them mycobacteria were seen in the stools. Histology exhibited acid fast bacilli in all of 11 cases it was performed, with duodenal and gastric biopsies largely represented (no. 6).

AIDS was the underlying condition in all patients, who were in overwhelming majority males (87%), with age ranging from 26 to 48 (average 34 years). They all presented advanced immunodeficiency with CD₄⁺ lymphocyte counts ranging from 1 to 65 per μ l (average 23.2, median 18) and were, or had been, treated with anti-retroviral drugs. Intravenous drug abusers largely predominated (54%), homosexuality (29%) and heterosexuality (17%) were the only other risk factors represented. Fever was the most common clinical sign (96%) followed by weight loss and anemia (Table 2). In no case the infection due to *M. genavense* was the case-defining pathology; all patients presented at least one other opportunistic infection

with *Candida* esophagitis (42%) and Cytomegalovirus retinitis (25%) being the most frequent.

Apart from two patients, who died when their cultures had not yet grown *M. genavense*, all subjects were treated with three or four antimycobacterial drugs: clarithromycin, amikacin, ethambutol, rifampin and ciprofloxacin were the most frequently used. The mean survival was 10.7 months but rises to 12.3 when two untreated patients and one, who died during the first week of therapy, are excluded; seven patients are still alive, two were lost at follow-up.

The frequency of disseminated *M. genavense* infections assessed, in three laboratories which yielded the majority (62%) of isolations, in comparison with the ones due to *M. avium* ranged from 3.3 to 6.8% with an overall percentage of 3.9.

Discussion

The source of the infection due to *M. genavense* in immunocompromised patient is unknown. The gastrointestinal route appears the most likely but no reservoir in the environment has been detected so far. Apart from humans, *M. genavense* has been found involved in lesions (mainly hepatomegaly, and thickening of the wall of the small intestine) in pet birds as documented in over 50 necropsies [10–18]. The isolation from a cervical lymph node of a dog [13] has also been reported.

The only isolations of *M. genavense* from HIV-seronegative patients refer to an immunosuppressed woman presenting a disseminated infection [19] and to a child with cervical lymphadenitis [20]. Species-specific DNA has been detected using PCR amplification in intestinal tissues of two out of nine asymptomatic subjects [21]. On the other hand a mycobacterium closely related to *M. genavense*, but differing from

Table 2. Percent frequency of symptoms in Italian and literature patients infected with *M. genavense*

Symptom	Italian patients	Literature patients
Fever	96	80
Anemia	62	25
Weight loss	62	48
Hepatomegaly	58	33
Abdominal pain	54	41
Splenomegaly	54	33
Adenomegaly	42	37
Pancytopenia	25	12
AIDS dementia complex	21	8
Wasting syndrome	12	18

it for three nucleotides in signature region of 16S rRNA gene, has been reported as cause of lymphadenitis in an immunocompetent woman [22].

Glancing over the literature concerning *M. genavense* infections in AIDS the number of cases may appear wider than it really is. In fact most of the cases reported in two collaborative studies [4, 23] have also been described separately [3, 24–31]. Once carefully screened, the overall number of cases fell, Italian ones excepted, to 69; individual information could be tracked down for 52 of them [4, 24–41], with remaining 17 being only included in a paper presenting cumulative data [23].

Wide is the geographic distribution of infections due to *M. genavense*, with Europe and North America yielding the majority of isolations; no case has however been described so far from Asia or Africa.

An almost complete overlapping characterizes patients from Italy and the ones reported in literature, with the only exception of risk factor: drug addiction (54%) largely prevailed in fact in the first group and the homosexuality (52%) in the second. The male to female ratio was 88% for Italian patients and 92% in literature, the mean age was 34 years in both and the mean CD₄⁺ lymphocyte counts were 23.2 and 21.4/μl, respectively.

A very close similarity was found in the two groups also for microbiological features; in fact, in literature too, *M. genavense* was isolated on average three times per patient, prevalently from blood (85% of patients) following a long incubation (average 40 days) in radiometric medium. Furthermore, in literature too, acid fast bacilli were seen in a great variety of tissues including lymph nodes, duodenal biopsies, spleen and liver; in many cases in such tissues PCR revealed *M. genavense* which subsequently failed to grow in culture.

Not substantially different is the ranking of symptoms within two groups (Table 2), the higher prevalences in Italians probably resulting from the systematic investigation we performed in records of such patients.

For what concerns opportunistic infections, the prevalence of *Cryptococcus meningitis*, Cytomegalovirus retinitis and *Candida esophagitis* were higher among Italian patients, while *Pneumocystis carinii* pneumonia was more frequent in reports from the literature.

A better survival seems to characterize our patients (10.7 versus 6.7 months) with seven of them, still alive and in good state of health, largely contributing to raise the average; the administration of anti-protease therapy, not available for previous patients, either Italian or from literature, may well explain such prolongation of survival. The comparison of therapeutic

regimens revealed in Italy a more frequent use of clarithromycin and amikacin. No correlation is possible between antimycobacterial treatment and survival as all our patients received at least three drugs; it should be noted, however, that in only one of them a temporary relapse occurred.

A 12.8% frequency of *M. genavense* among the total mycobacterial disseminated infections had been estimated by Swiss HIV Cohort Study [23]; our attempt to assess this frequency in Italy in comparison with *M. avium* yielded a value of 3.9%, very close to 3.8 and 4% reported by others [9, 24]. We consider, however, such value to underestimate the reality as only *M. genavense* grown in culture fell under our observation while not infrequent, for this species, are growth failures as shown by high rate of cases detected by PCR only [29, 30, 42].

The worldwide rise of frequency of *M. genavense* isolations is certainly related both to an increased awareness and to the diffusion of use of liquid culture media; nevertheless, because of the difficulties in detecting this agent, a correct diagnosis is still achieved only in a limited number of cases. In consideration of its unquestionable clinical importance, a disseminated infection due to *M. genavense* represents a possibility to take into account mainly in presence of not otherwise explained symptoms like fever, weight loss, hepatomegaly and abdominal pain.

The extreme rarity, in non-HIV infected patients, of human diseases due to *M. genavense* seems to suggest a low virulence for this organism which however, once ingested, may colonize the gut [21]. Recent report of isolations outside of AIDS may however rise some caution all the more as microscopic observation of mycobacteria which fail to grow in culture is not exceptional also in immunocompetent subjects.

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