ORIGINAL ARTICLE

Trend Reversal in the Frequency of Mycoses in Hematological Neoplasias

Autopsy Results From 1976 to 2005

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SUMMARY

Introduction: Fungal infections of internal organs are a major complication for patients with hematological neoplasias. For more than 20 years, the frequency of such mycoses has been increasing with the aggressiveness of tumor treatment.

Methods: Autopsy findings over a 30-year period (1976 to 2005) from a single institution (Institute of Pathology, University of Essen) were retrospectively classified according to basic disease, frequency of mycoses, kind of mycoses, organs involved, hematopoietic transplantation, and cause of death.

Results: 340 of 1591 autopsied patients with hematological neoplasias (21.4%) revealed an invasive mycosis. The proportion increased from about 10% before 1980 to some 30% in the 1990s but fell to 21% by 2005. The frequency of mycoses decreased significantly both for transplanted patients (from 47.5% to 30.3%) and for non-transplanted patients (from 29.8% to 16.4%). The rate of deaths due to mycosis also decreased. The relative frequency of candidal mycoses went down, while aspergilloses predominated. The organ most frequently involved was the lung.

Discussion: The autopsy results signal a trend reversal in the leading complication of the treatment of hematological neoplasias and lend support to the assumption that antimycotic strategies are having a positive effect.

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Key words: mycosis, leukemia, lymphoma, autopsy, hematological neoplasia

Fungal infections of the internal organs are a diagnostic and therapeutic problem which has been known for many years. This mostly affects patients with immune suppression, particularly after organ transplantation or intensive cytostatic chemotherapy. Hematological neoplasias are particularly important in this context, as a result of either the primary underlying disease or the aggressive treatment required. Severe mycotic complications in patients with hematological neoplasias have led to the development of guidelines for fungus diagnosis (1, 2). Moreover, in recent years, treatment has been started as soon as possible with newly developed antimycotics, such as fluconazole, voriconazole, liposomal amphotericin B, caspofungin or posaconazole (3, 4, 5). In this context, it is of interest to establish whether autopsy results might reflect the success of this antimycotic treatment or whether invasive mycoses have continued to increase – as was found in earlier autopsy studies (6, 7, 8).

Methods

From 1976 to 2005, a total of 11 859 autopsies were performed in the Institute of Pathology, Essen University Hospital. The archived documents on these autopsies include

- applications for clinical dissections, with information on basic disease, treatment, the clinical course, and the clinical cause of death,
- macroscopic autopsy records, giving the basic disease, complications, secondary diseases, and causes of death, as well as
- reports on histological findings.

Deceased patients with hematological neoplasias were selected and the cases classified according to the following diagnostic groups:

1. Acute leukemias
2. Chronic myeloproliferative diseases (CMPE)
3. Non-Hodgkin's lymphomas of low grade malignancy ("low grade NHL")
4. Non-Hodgkin's lymphomas of high grade malignancy ("high grade NHL")
5. Hodgkin's lymphomas
6. Multiple myelomas.

With the exception of 10 cases, the hematological neoplasia had been verified during life by histology or histopathology of the bone marrow or on lymph node

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FIGURE 1

Five-year frequency of invasive mycoses in hematological neoplasias. Significant group differences and 95% confidence intervals (mean 21.4%; 340 of 1591 patients).

FIGURE 2

Frequency of deep mycoses in different neoplasias. Changes with time

biopsies. The classification was in accordance with the last intravital diagnosis. Cases with primary bone marrow aplasia were excluded. Clinical diagnoses of fungal infections or of suspected fungal infections were excluded. Allogenic and autologous transplantation of bone marrow or hematopoietic stem cells were assigned to the separate group of "hematological transplantations" (HT).

The autopsy was normally a three cavity operation. The thoracic and abdominal organs were investigated in every evaluated case. The histological findings were made on paraffin sections stained with hematoxylin-eosin. In cases where a fungal infection was suspected by the clinician or by the pathologist – for example, with necroses –, a periodic acid Schiff reaction was performed, or (more rarely) silver staining with the Grocott method. On the basis of the histological findings, the fungi were classified as yeasts (either *Candida*-like mycoses or cryptococcoses) or filamentous fungi (either *Aspergillus*-like mycoses, including *Fusaria*, or zygomycoses). Double mycoses and unclassifiable mycoses were registered separately.

The essential cause of death was analyzed on the basis of the overall findings. For this purpose, the severity and extension of the invasive mycosis was weighted, as well as the severity of bacterial infections and bleeding complications. Other causes of death, such as the progressive character of the basic disease, a graft-versus-host reaction, and pneumonia were subsumed in a separate group.

The statistical evaluation was predominantly descriptive and covered the frequency of autopsy, the frequency of mycosis, type of mycosis, basic disease, organ involvement, and causes of death. The analysis was performed with the program SPSS version 12.0 for Windows. The Pearson chi-square test was used to determine the significance of differences between the five-year fre-

quencies. The confidence intervals were calculated with the SAS program.

Results

At the Essen University Institute of Pathology, invasive mycoses were a frequent and severe complication in patients with fatal hematological neoplasias. Thus 340 of 1591 autopsied patients with hematological neoplasias (21.4%) exhibited a deep mycosis. There were major differences in the percentages of mycosis from year to year. The values increased from about 15% at the beginning of the 1980s to about 30% in 2000, with peak values at 40%. The frequency was less from 2001 to 2005, with a mean of 21%. The comparison of five-year periods (figure 1) showed that there was an increase in invasive mycoses up to about 1995, followed by a slight and then marked increase in these complications. The increase was significant for two of the five-year periods. The decrease did not reach significance for the overall group, although the decreases were significant for the separate groups of the non-HT cases (figure 4) and the special risk group of the HT patients (figure 3).

As expected, the group of acute leukemias was most strongly affected by the complication of invasive mycosis, with 29.6% of the cases (167 of 565). Chronic myeloproliferative diseases exhibited this complication in 21.1% of cases (59 of 280 cases). Aggressive non-Hodgkin's lymphomas exhibited mycosis in 18.1% of cases (56 of 309 cases), while 17.5% (38 of 217) cases of indolent non-Hodgkin's lymphoma were affected. 11 of 106 cases (10.4%) of Hodgkin's lymphoma and 9 of 114 cases of multiple myeloma (7.9%) exhibited invasive mycosis at autopsy.

The frequency of mycosis increased to a greater or lesser extent for all neoplasias (figure 2). While the value for the chronic myeloproliferative diseases stabilized at about 20% by 2005, there has been a moderate decrease in the last 5 to 10 years in the rate of mycotic complications in patients with acute leukemia and lymphomas.

The highest rate of mycoses was found in patients after transplantation of either bone marrow or hematopoietic stem cells (figure 3), with a mean value of 36% (96 of 267 patients). The initial value was high, even in the 1980s, followed by a peak of 47.5% in the 1990s and a significant decrease to 30% in the period 2001 to 2005 (figure 3).

The mycosis rate in non-transplanted patients with hematological neoplasias had a lower overall value than after transplantation (18.4%, 244 of 1324 patients). This value increased significantly until 1990, but then decreased, more quickly than with the HT cases ($p = 0.003$) (figure 4).

The histological classification of the invasive mycoses found 193 cases of filamentous fungi, including 179 aspergilloses and 14 zygomycoses. In comparison, there were 109 cases of yeast infection, 107 cases of candidosis, and 2 cases of cryptococcosis. Over the course of years, it was striking that candidoses decreased relative to aspergilloses (table 1). 25 cases exhibited double mycoses. In 13 cases, the fungal structures could not be classified.

The lungs were the predominant organ affected. This was particularly with *Aspergillus*, but also often with *Candida*. Candidoses were dominant in the gastrointestinal tract. Kidneys, myocardium, endocardium, brain, and liver were much more rarely affected, mostly in the context of fungal sepsis. Thus, in 40% of mycoses (134 cases), at least two non-adjacent organs were simultaneously involved (table 2).

The causes of death were determined on autopsy and showed a significant increase in fatal mycoses, up to a peak value of 30% in 2000, coupled to a decrease in bleeding complications. Within the last five years, fatal mycoses fell to 21%. Fatal bacterial complications and other non-mycotic causes of death, such as progression of the basic disease, interstitial pneumonia and rejection reactions, remained about constant over the years (table 3). In 23 of the 25 patients with double mycosis, this was the cause of death.

For the period 1976 to 1983, the clinical diagnosis or suspicion of invasive mycosis during life corresponded to 30% of the mycoses established on autopsy. Within the period 1998 to 2005, the proportion of clinically diagnosed cases rose to 42%. The proportion of clinically undiagnosed cases fell correspondingly from 70% to 58%. In the period 1998 to 2005, the preterminal clinical diagnosis of severe mycosis was not confirmed on autopsy of 13 patients.

Discussion

The proportion of invasive mycoses in unselected autopsies was about 1% up to 1970 (9), increasing to about 5% in the 1990s (6). Between 2001 and 2005 in the Essen

Five-year mycosis rate after hematological transplantations. Significant group differences and 95% confidence intervals (mean 36%; 96 of 267 patients).

Institute, there was a mean of 6.1% mycoses – 57 cases in all 935 autopsies performed. In comparison to this, the mean mycosis rate is greater by a factor of 3 to 6 in patients with underlying hematological disease (10, 11).

Autopsy data are of high validity. However, epidemiological statements are in principle restricted, as the autopsies are a non-representative sample, dependent on changes in clinical speciality, different indications for autopsy, and differences in approval procedures. We nevertheless wish to point out that hematological neoplasias were frequent in the Essen West German Tumor Center throughout the period, that the transplantation rate has been high since 1981, and that all autopsies within the period of observation required standard approval.

What may be limiting is the decrease in autopsy numbers, which may mask a bias in the results. In accordance with generalizing developments, the number of autopsies has been decreasing from 3449 for the period 1976 to 1980 to 2017 for the period 1986 to 1990 to 935 for the period 2001 to 2005. On the other hand, there was relatively little change in the proportion of hematological neoplasias in the group with mycotic complications (figure 1). While the percentage of hematological neoplasias in the autopsies increased from 9.9% (1976 to 1980) to 16.4% (1991 to 1995), the mycosis rate increased from 11.1% to 31.4% within the same period. The decrease in the autopsies with hematological neoplasias from 16.4% (1991 to 1995) to 10.7% (2001 to 2005) was relatively lower than the decrease in the mycosis rate from 31.4% to 13.1% (1996 to 2005) to 16.4% (2001 to 2005).

Given these limitations, the Essen results should be confirmed with additional studies, particularly as previous publications have only reported increases in mycotic complications. In any case, a study with a similar number of autopsied patients with hematological

Mycosis rate in non-transplanted patients with hematological neoplasias. Significant group differences and 95% confidence intervals (mean 18.4%; 244 of 1324 patients).

neoplasias found no increase, but stagnation in the rate of mycoses for the period 2000 to 2003 (8) – a result which might also indicate a trend reversal. It should also be tested if antimycotic effects are also detectable for other risks, such as organ transplantation or intensive medical care.

Data on fungal infections in living patients are unreliable for epidemiological purposes, as a clinical suspicion can often not be confirmed with microbiological or histological test procedures. Moreover, fungal infections are not notifiable. In this context, results from autopsies are of considerable weight, despite the reservations, as they combine the histological detection of invasive mycosis with the demonstration of pathogenicity, based on the patient's reactions during life. This is especially true of the present data, which are based on a large number of cases from a single institution, largely standardized investigation and documentation. The results were based on the histomorphological confirmation of mycosis, which could be classified in most cases as a filamentous fungus or a blastomyces. It was usually possible to make additional distinctions, for example, between zygomycosis and cryptococcosis. Nevertheless, further histological subclassification, such as the distinction between *Aspergillus* and *Fusaria*, was limited and necessitated microbiological analyses, which again are somewhat unreliable with material from autopsies, particularly after antimycotic therapy.

The results show that the decade long increase in the rate of mycosis in hematological neoplasias has been successfully stopped. This applies both to patients without transplantation and to patients who had received bone marrow or stem cell transplantations. Even though the number of autopsied HT patients has decreased during the last five years, there has been an overproportional decrease in mycotic complications. While in the period

1996 to 2000 almost every second transplanted patient (38 of 80 autopsied patients) exhibited a mycosis, in spite of the increase in autologous transplantation, this complication only affected every third transplanted patient (10 of 33 autopsies) in the period 2001 to 2005. Within this period, the number of first transplants at Essen University Hospital remained at the same high level. This applied to both autologous and allogenic stem cell transplantation (about one third and about two thirds, respectively) (12). The assumption that the decrease in mycosis rate is solely due to the increase in autologous transplantations is not compatible with the decrease in the rate of mycoses even in non-transplanted patients. Moreover, published clinical data from the Essen Transplantation Clinic also indicate that antimycotic measures have had a positive effect (13, 14).

The rate of mycoses depends on the type of basic disease and its treatment, particularly any immunosuppressive measures. Impaired leukocyte function or granulocytopenia in acute leukemia increase the risk of fungus, even without aggressive treatment. In diseases in which bone marrow function is maintained over long periods, such as multiple myeloma, there is seldom correlation with mycosis. The rate of mycosis in multiple myeloma in our group of patients only increased with the introduction of hematopoietic stem cell transplantation. Only 4 of 103 myeloma patients without transplantation (3.9%) exhibited fatal mycosis, in comparison with 5 of 11 (45.5%) of transplanted myeloma patients. The rate of mycoses in the other disease groups increased particularly during the 1990s, which probably also reflects the more aggressive forms of treatment. In these cases too there was no detectable increase for the period 2001 to 2005, admittedly with decreases in the numbers of autopsies. HIV infection was only given for four patients, implying that this is not a relevant factor for the results.

It was striking that candidoses decreased relative to aspergilloses over the years. This development has been found in other studies (15, 16, 17) and may be of some significance for radiological diagnosis. The reason is that good images of aspergillosis infections are easier to obtain, as they are typically accompanied by focuses some centimeters in size. In contrast, the focuses in

TABLE 1

Frequencies of *Aspergillus* and *Candida* mycoses in hematological neoplasias

Period	<i>Aspergillus</i> (n)	%	<i>Candida</i> (n)	%
1976–1980	10	28	26	72
1981–1985	41	56	32	44
1986–1990	36	60	24	40
1991–1995	51	71	21	29
1996–2000	33	66	17	34
2001–2005	17	89	2	11
Total	188	61	122	39

TABLE 2

Organ involvement in deep mycoses

	Case number		Filamentous Fungi		Blastomycetes		Undefinable n
	n	%*1	Aspergillosis n	Zygomycosis n	Candidosis n	Cryptococcosis n	
Lung	244	72.8	173	10	50	2	9
Oesophagus, gastrointestinal tract	154	46.0	22	2	127	1	2
Liver	46	13.7	18	3	24	1	0
Spleen	33	9.9	17	3	17	1	0
Kidney	74	22.1	33	8	31	1	1
Heart	68	20.3	44	2	22	0	0
Brain	48	14.3	35	7	6	0	0
Other	51	15.2	27	3	17	2	2
Fungal sepsis*2	134	40.0	71	10	50	2	1

*1 Percentage of total number, n = 340

*2 Fungal sepsis, involvement of two or more non-adjacent organs

TABLE 3

Causes of death at autopsy in 1591 patients with hematological neoplasias

Period		1976–1980	1981–1985	1986–1990	1991–1995	1996–2000	2001–2005
Patients (n)		342	402	309	274	164	100
Mycoses	(in percent)	7	14*1	18*1	29*1	30	21
Bacterial infections		14	10	12	16	18	16
Hemorrhage		22	20	22	13*2	8	14
Other		56	55	48	42	44	49

*1 Increase p < 0.05

*2 Decrease p < 0.05

candidoses are in the millimeter range and are therefore more difficult to detect. No increases in zygomycosis were found (18).

Invasive mycoses are associated with a high death rate in patients with hematological neoplasias, at 30% for candida mycoses and 40% for aspergillosis (19). According to our autopsy results, the percentage of deaths associated with invasive mycosis increased up to 30% in the 1990s, followed by a decrease to about 20% in recent years (table 3). This reduction in death associated with mycosis is an additional indication of the favorable effects of the antimycotic measures (20).

The death rate due to hemorrhage decreased; deaths from bacterial infections remained about constant. "Other causes" of death included disease progression, interstitial pneumonia, graft-versus-host disease (GVHD), and viral infections. As specific tests were performed for mycosis in all cases and histological studies were performed, especially in the lungs, fatal mycosis can largely be excluded for this group.

Nevertheless, the results also show that the complication rate is still high, so that antimycotic therapy must be

consistently implemented and continued, even if new generations of antimycotic drugs demand additional financial resources (21). This expenditure is justified by the need to master the most important complication induced by potentially curative therapy with immune suppression.

Finally, our results show that clinical autopsies can provide valuable knowledge for doctors, patients and relatives. This is not only for the individual case, but also for the development of the disease and for diagnostic and therapeutic measures. Clinical autopsy is a simple and highly effective instrument for quality assurance and has been too little used for this purpose for many years.

This article is dedicated to Professor L.-D. Leder on the occasion of his 75th birthday. Professor Leder was Director of the Pathology Institute at Essen University Hospital from 1975 to 1998.

Conflict of interest statement

The authors declare that no conflict of interest exists according to the guidelines of the International Committee of Medical Journal Editors.

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