**Supplemental Materials**

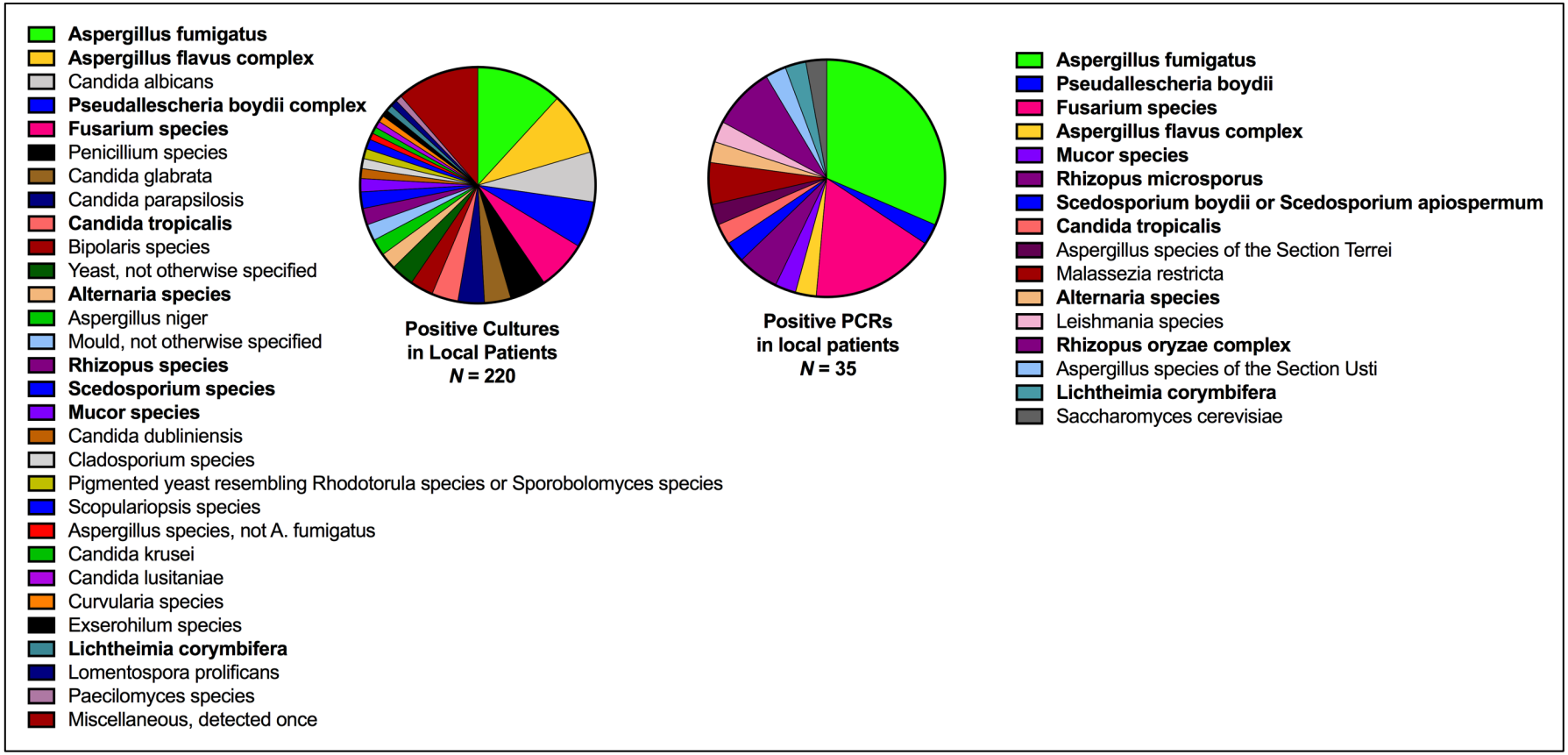
Supplemental Methods

Determination of Organism Prevalence

For all local patients with positive results, unique medical record-date combinations were determined and results by broad-range fungal PCR or fungal culture determined. For each organism identified the percent of total culture or PCR results, respectively, was calculated. For culture, the numbers of isolates for which sequence-based identification was performed and those isolates for which no identification was possible were recorded. Organisms identified only once were grouped into a single category.

**Supplemental Results**

There were 15 positive cultures from 15 unique patients identified with preliminary TATs longer than 300 hrs. Fourteen of these came from local patients, only three of whom were inpatients: two collecting in the operating rooms and one in the post-anesthesia care unit. An additional culture was collected in the pre-operative care unit, although this patient was designated an outpatient. None of these patients met inclusion criteria for chart review. The preliminary results from these outlier cultures comprised 6 unidentified organisms that required further incubation or sequence identification, two cultures that were positive for *Bipolaris spp*, and one culture each with *Exophiala spp*, *Exserohilum spp*, presumptive *Fusarium*, *Cladosporium spp*, *Pseudoallecheria boydii*, *Pleurostomophora richardsiae*, and one culture with both *Penicllium* and *Acremonium*. One mould could not be isolated from contaminating bacteria. Of the 9 cultures in which the number of colonies recovered was recorded, 7 had only one colony.




**Supplemental Figure S1**: Relative prevalence of fungal organisms detected by PCR and Culture from Local Patients.
The relative abundance of detected fungal organism by culture (left) and broad-range PCR (right) in all local patients, including those with chart review. Results were deduplicated to include only unique MRN-date combinations. Organisms detected by both methods are in bold with colors consistent across charts. Organisms detected only once by culture are grouped in a single category (*N* = 25 / 220 isolates). All *Fusarium spp* are grouped into a single category. Culture was unable to determine the identity of an organism for *N* = 3 isolates and sequence-based identification was required for *N* = 25 / 220 isolates. Our laboratory has detected *Saccharomyces cerevisiae* DNA by broad-range fungal PCR in multiple lots of Eswabs and report this finding with a disclaimer indicating a possible source of pre-analytical contamination. Parasites like *Leishmania spp* are occasionally detected by broad-range fungal PCR due to homology of the ribosomal gene within eukaryotes and are reported as an incidental finding.

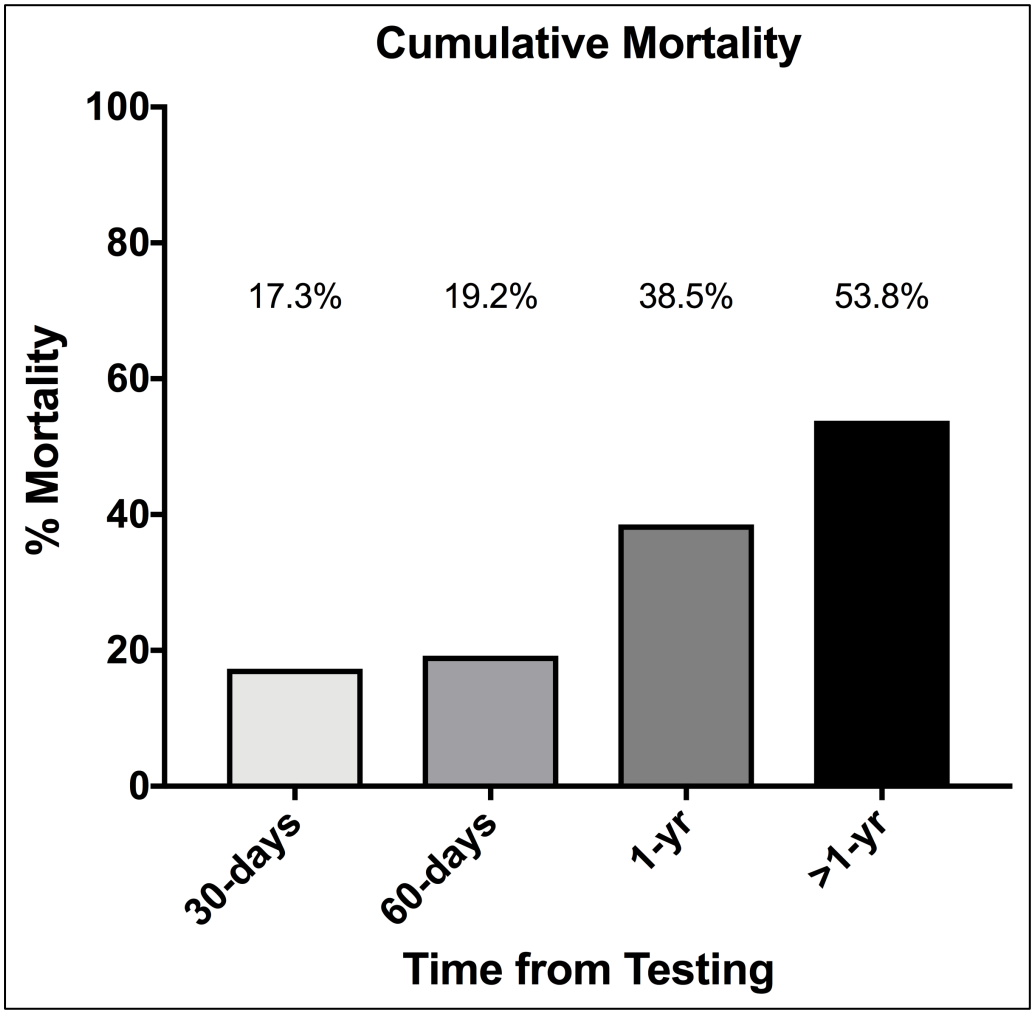
**A**

| Sensitivity | Culture | PCR | Culture + PCR | Direct Stain | Frozen Section | Both Stains |
|---|---|---|---|---|---|---|
| **Culture** | - | 0.1136 | **0.0269** | **0.0486** | 1.0 | 0.4136 |
| **PCR** | 0.1136 | - | 0.7370 | **0.0002** | 0.1375 | 0.5321 |
| **Culture + PCR** | **0.0269** | 0.7370 | - | **< 0.0001** | **0.0302** | 0.2972 |
| **Direct Stain** | **0.0486** | **0.0002** | **< 0.0001** | - | 0.0872 | **0.0068** |
| **Frozen Section** | 1.0 | 0.1375 | **0.0302** | 0.0872 | - | 0.5472 |
| **Both Stains** | 0.4136 | 0.5321 | 0.2972 | **0.0068** | 0.5472 | - |

**B**

| Specificity | Culture | PCR | Culture + PCR | Direct Stain | Frozen Section | Both Stains |
|---|---|---|---|---|---|---|
| **Culture** | - | 1.0 | 1.0 | 0.4831 | 1.0 | 1.0 |
| **PCR** | 1.0 | - | 1.0 | 0.4831 | 1.0 | 1.0 |
| **Culture + PCR** | 1.0 | 1.0 | - | 0.2246 | 1.0 | 1.0 |
| **Direct Stain** | 0.4831 | 0.4831 | 0.2246 | - | 0.4000 | 0.4000 |
| **Frozen Section** | 1.0 | 0.1375 | 0.0302 | 0.4000 | - | 1.0 |
| **Both Stains** | 1.0 | 1.0 | 1.0 | 0.4000 | 1.0 | - |

**Supplemental Figure S2. Statistical comparison of sensitivities and specificities for each fungal assay.**
Results of Fisher's Exact test are reported as p-values for pairwise comparisons of ***A)*** sensitivities and ***B)*** specificities for fungal culture, broad-range fungal PCR, PCR and culture submitted in tandem, direct stain, frozen section, or direct stain and frozen section performed in tandem.




**Supplemental Figure S3**: **Cumulative mortality for local patients with parallel fungal PCR and cultures.**
Patient status as alive or dead at time of chart review and, if applicable, date of death was determined for the 52 patients included for chart review. Time to death from fungal testing was determined and binned as 0 - 30 days; 31 – 60 days; 61 – 365 days, or greater than 1 year and used to calculate cumulative mortality presented in the figure.

**Supplemental Table S1: Total Specimens and Positivity Rates by Assay, Source Population, and Anatomic Site**

| Group | Unique Patients | Culture *N* (patients) | Culture % pos | PCR *N* (patients) | PCR % pos | Cx vs PCR p-value[1] |
|---|---|---|---|---|---|---|
| All Sinus | 1,459 | 1446 (949) | 13.7% | 644 (569) | 37.1% | < 2.2e-16 |
| Ref. lab | 529 | 50 (31) | 12.0% | 543 (498) | 35.9% | < 0.0005 |
| Local Patients | 930 | 1396 (918) | 13.8% | 101 (71) | 43.6% | 3.73e-12 |

[1]Fisher's exact test.

**Supplemental Table S2: Chart review data for all included patients with parallel fungal culture and broad-range PCR**

| Patient Number | Underlying Diagnosis | Pre-Test EORTC Category | Antifungal Exposure Prior to Specimen Collection | PCR Result | Culture Result | Direct Stain Results | Frozen Section Results | Post-Test Assessment of Fungal Disease | Clinical Interpretation of Results |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Acute lymphocytic leukemia, relapsed, s/p G-CLAM | Probable | fluconazole prophylaxis; posaconazole treatment | *Alternaria sp* | NEG | No fungal elements | Hyphal elements, favor septate | Fungal etiology | True Positive |
| 2 | Acute myelogenous leukemia, refractory, s/p G-CLAM | Proven | posaconazole prophylaxis; amphotericin B | *Fusarium fujikuroi* species complex | *Fusarium oxysporum* | 2+ hyphal elements | Tissue invasive fungal hyphae and rare yeast forms | Fungal etiology | True Positive |
| 3 | Diabetes mellitus, type II | Proven | posaconazole; amphotericin B | *Rhizopus oryzae* complex | *Rhizopus oryzae* complex | Few hyphal elements | Suspicious for fungal organisms | Fungal etiology | True Positive |
| 4 | Large B-cell lymphoma; history of proven orbital aspergillosis | Possible | oral antifungals, not specified | NEG | NEG | No fungal elements | Atypical lymphoid cell proliferation | Other etiology | True Negative |
| 5 | Diabetes mellitus, type II | Proven | amphotericin B | *Aspergillus fumigatus* | *Aspergillus fumigatus* | Many hyphal elements | *Not performed* | Fungal etiology | True Positive |
| 6 | aplastic anemia, s/p matched related bone marrow transplant | Probable | isavuconazole, voriconazole | NEG | NEG | No fungal elements | Inflamed granulation tissue | Fungal etiology | False Negative (degraded hyphae seen on final histopathology) |
| 6 | aplastic anemia, s/p matched related | Proven | amphotericin B | *Aspergillus fumigatus* | NEG | moderate hyphae | Tissue invasive fungal hyphae | Fungal etiology | True Positive |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | bone marrow transplant | | | | | | | | |
| 6 | aplastic anemia, chemotherapy prior to matched related bone marrow transplant | Probable | voriconazole | NEG | NEG | No fungal elements | Negative for invasive fungal elements | Fungal etiology | False Negative |
| 7 | Acute myelgenous leukemia, relapsed, s/p CLAM | Probable | isavuconazole, amphotericin B | *Fusarium solani* | Presumptive *Fusarium solani* | No fungal elements | Positive for fungal elements | Fungal etiology | True Positive |
| 8 | chronic myelogenous leukemia with blast crisis | Proven | isavuconazole prophylaxis | *Lichtheimia corymbifera* | *Lichtheimia corymbifera* | Many hyphal elements | Fungal hyphae with necrotic debris | Fungal etiology | True Positive |
| 9 | Acute myelgenous leukemia, relapsed, s/p G-CLA | Proven | micafungin, amphotericin, isavuconazole prophylaxis | *Fusarium fujikuroi* | *Fusarium fujikuroi* | Many hyphal elements | No definite fungal elements | Fungal etiology | True Positive |
| 10 | Chronic lymphocytic leukemia | Possible | amphotericin B | NEG | NEG | No fungal elements | No fungal elements identified | Other etiology | True Negative |
| 11 | myelofibrosis, s/p PBSCT | Probable | voriconazole | *Aspergillus fumigatus* | NEG | No fungal elements | Fungal hyphae | Fungal etiology | True Positive |
| 12 | Acute lymphocytic leukemia, s/p non-myeloablative HSCT | Proven | none | *Rhizopus oryzae* complex | *Rhizopus sp.* | No fungal elements | Positive for fungal elements | Fungal etiology | True Positive |
| 13 | History of afrin & intranasal acetominophen | Not Applicable | none | *Candida tropicalis* | *Candida tropicalis*, *Scopulariopsis brevicaulis* | Moderate fungal elements | Suspicious for invasive fungal hyphae | Other etiology | Bystander/colonizer |
| 14 | Sinonasal squamous cell carcinoma, s/p chemotherapy & radiotherapy | Proven | fluconazole | *Aspergillus fumigatus* | *Aspergillus fumigatus* | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 15 | Acute myelogenous leukemia, s/p G-CLAM | Possible | fluconazole, amphotericin B | NEG | NEG | No fungal elements | *Not performed* | Other etiology | True Negative |
| 16 | Acute myelogenous leukemia, s/p PBSCT | Probable | amphotericin B, micafungin | *Aspergillus sp* of Section Terrei | *Aspergillus terreus* | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 17 | Intra-abdominal sepsis; History of | Probable | none | NEG | *Scedosporium apiospermum* complex | No fungal elements | Negative for fungal elements | Fungal etiology | True Positive |

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|---|---|---|---|---|---|---|---|---|---|
| | liver and kidney transplant | | | | | | | | |
| 18 | Acute myelogenous leukemia, receiving chemotherapy | Proven | none | *Aspergillus flavus* complex | NEG | No fungal elements | Positive, appear [to be] septate hyphae | Fungal etiology | True Positive |
| 19 | Acute myelogenous leukemia | Proven | posaconazole | *Fusarium solani* | *Fusarium solani* | Many fungal elements | Positive for fungal forms | Fungal etiology | True Positive |
| 20 | Acute myelogenous leukemia, s/p MEC; concurrent A. fumigatus pneumonia (antecedent dx) | Proven | Isavuconazole, micafungin | *Aspergillus fumigatus* | *Aspergillus fumigatus* | Many fungal elements | Necrotic tissue with fungus. Viable tissue suspicious for fungal forms. | Fungal etiology | True Positive |
| 21 | Acute lymphocytic leukemia, s/p rituximab and cytarabine | Proven | fluconazole prophylaxis; amphotericin B | *Mucor sp* | *Mucor sp* | Many fungal elements | Numerous fungal hyphae | Fungal etiology | True Positive |
| 22 | Myelodysplastic syndrome, s/p guadecitabine | Probable | amphotericin B | *Aspergillus fumigatus* | *Aspergillus fumigatus* | Occasional fungal elements | Negative for fungal elements | Fungal etiology | True Positive |
| 23 | Acute myelogenous leukemia, s/p G-CLAM | Proven | voriconazole, amphotericin B | *Fusarium fujikuroi* | *Fusarium sp* | Many fungal elements | Positive for fungal organisms, appear angioinvasive | Fungal etiology | True Positive |
| 24 | Chronic lymphocytic leukemia, s/p CAR-T cell infusion | Probable | none | *A. fumigatus* | NEG | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 25 | Aplastic anemia s/p haploidentical bone marrow transplant | Probable | voriconazole, micafungin, amphotericin B | *Rhizopus microsporus* or *Rhizopus azygosporus* | *Rhizopus sp* | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 26 | Acute myelogenous leukemia, multiple myeloma | Probable | Amphotericin B | Rhizopus oryzae | NEG | No fungal elements | No definite fungal organisms | Fungal etiology | True Positive |
| 27 | Acute myelogenous leukemia | Proven | Amphotericin B | *Mucor circinelloides* | *Mucor sp* | Many fungal elements | *Not performed* | Fungal etiology | True Positive |
| 28 | Acute myelogenous leukemia | Possible | posaconazole, amphotericin B | NEG | NEG | No fungal elements | No fungal forms identified | Other etiology | True Negative |
| 29 | Acute myelogenous leukemia, type 1 | Possible | posaconazole | NEG | NEG | No fungal elements | *Not performed* | Other etiology | True Negative |

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|---|---|---|---|---|---|---|---|---|---|
| | DM, IVDU, endocarditis | | | | | | | | |
| 30 | Acute myelogenous leukemia, relapsed s/p FLAG | Probable | amphotericin B | *Rhizopus microsporus* or *Rhizopus azygosporus* | *Rhizopus sp* | No fungal elements | Fungal hyphae present; at least focally invasive | Fungal etiology | True Positive |
| 31 | Acute lymphocytic leukemia, s/p CAR-T infusion | Possible | micafungin | NEG | NEG | No fungal elements | No definite fungal forms | Other etiology | True Negative |
| 32 | Acute myelogenous leukemia, relapsed | Proven | amphotericin B | *Scedosporium boydii* or *S. apiospermum* | *Pseud-allecheria boydii* complex | No fungal elements | Tissue invasive fungal hyphae seen | Fungal etiology | True Positive |
| 33 | Acute myelogeneous leukemia, s/p matched PBSCT | Possible | voriconazole prophylaxis | NEG | NEG | No fungal elements | Rare hyphae reported; determined to be diagnostic error; treated as positive result for culture and PCR concordance. | Other etiology | True Negative |
| 34 | Acute lymphoctic leukemia | Possible | voriconazole, amphotericin B | *Aspergillus flavus* | *Aspergillus flavus* | No fungal elements | Positive for fungal forms with angioinvasion | Fungal etiology | True Positive |
| 35 | Acute lymphoctic leukemia, s/p cord blood transplant | Possible | posaconazole prophylaxis | NEG | NEG | No fungal elements | *Not performed* | Other etiology | True Negative |
| 36 | Acute myelogenous leukemia, evolved from myelofibrosis | Possible | posaconazole prophylaxis, amphotericin B | NEG | NEG | No fungal elements | Rare possible fungal hyphae; no tissue, mucus only; bacterial forms | Other etiology | True Negative |
| 37 | Cutaneous T-cell lymphoma with HLH | Proven | none | *Aspergillus fumigatus* | *Aspergillus fumigatus* | Moderate hyphae elements | fungal hyphae | Fungal etiology | True Positive |
| 38 | Myelofibrosis | Probable | voriconazole prophylaxis, capsofungin | *Pseud-allescheria boydii* | *Pseud-allescheria boydii* complex | Moderate fungal elements | No evidence of fungal infection | Fungal etiology | True Positive |
| 39 | HIV (on HAART) | Not Applicable | None | NFDD | *Pencillium sp* AND *Cephalotheca sulfurea* OR | 2+ Yeast seen | *Not performed* | Other etiology | Bystander/colonizer |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Phialemonium inflatum* | | | | |
| 40 | Acute lymphocytic leukemia, salvage chemotherapy; concurrent fungal pneumonia (A. fumigatus, Fusarium spp) | Proven | posaconazole | *Fusarium sp* | *Fusarium sp* | Moderate fungal elements | Tissue invasive fungus present; "size of organisms suggests zygomycetes" | Fungal etiology | True Positive |
| 41 | Diabetes mellitus type II, distant history of chraniopharyngioma and CSF leak | Not Applicable | None | NEG | NEG | No fungal elements | *Not performed* | Other etiology | True Negative |
| 42 | Acute myelogenous leukemia, relapsed; history of sinus aspergillosis | Possible | voriconazole prophylaxis, isavuconazole | NEG | NEG | No fungal elements | No definite fungal organisms | Other etiology | True Negative |
| 43 | Acute myelogenous leukemia (secondary to DLBCL treatment), sp chemotherapy | Possible | amphotericin B | NEG | *Aspergillus fumigatus* | No fungal elements | No definitive fungal forms; *recent prior positive result* | Fungal etiology | True Positive |
| 44 | Myelodysplastic syndrome, s/p PBSCT | Possible | voriconazole | *Aspergillus ustus* and *Scopulariopsis brevicaulis* | *Aspergillus ustus* complex and *Scopulariopsis brevicaulis* | Moderate hyphal elements | *Not performed* | Fungal etiology | True Positive |
| 45 | Liver transplant, plasma cell hepatitis, concurrent SSTI with Scedosporium spp | Probable | voriconazole | *Scedosporium boydii* or *Scedosporium apiospermum* | *Pseud-allescheria boydii* complex | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 46 | Acute lymphocytic leukemia, s/p CAR-T infusion; fungal elements on histopathology (PCR on FFPE positive for A. fumigatus months later, no paired culture with that specimen) | Proven | amphotericin B, posaconazole | NEG | NEG | No fungal elements | *Not performed* | Fungal etiology | False Negative |

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|---|---|---|---|---|---|---|---|---|---|
| 47 | Acute lymphocytic leukemia, s/p chemotherapy | Proven | fluconazole prophylaxis, amphotericin B | *Aspergillus fumigatus* | *Aspergillus fumigatus* | Few hyphal elements | *Not performed* | Fungal etiology | True Positive |
| 48 | Acute myelogenous leukemia, secondary | Possible | posaconazole prophylaxis | *Aspergillus fumigatus* | NEG | No fungal elements | *Not performed* | Fungal etiology | True Positive |
| 49 | B-cell lymphoma (lmited specimens for both culture & PCR) | Proven | none | NEG | NEG | No fungal elements; *only swab submitted* | *Not performed; recent prior positive for septate hyphae in decalcified specimen* | Other etiology | False Negative |
| 50 | Intracranial abscess, meningitis, and bacteremia due to Staphylococcus aureus with sinus involvement | Not Applicable | none | *Malassezia restricta* | NEG | No fungal elements | No apparent fungal organisms | Other etiology | Bystander/colonizer |
| 51 | EtOH abuse and ESRD with marked anemia | Proven | none | *Rhizopus microsporus* | NEG | No fungal elements | No obvious fungal forms or significant inflammation | Fungal etiology | True Positive |
| 52 | HIV, AIDS (CD4 14 cells/mcL) | Not Applicable | amphotericin B | *Aspergillus fumigatus* | NEG | No fungal elements | *Not performed* | Fungal etiology | True Positive |

**Supplemental Table S3:**

| **Patient Number** | **Did Fungal PCR Drive Change in Management in True Positive Cases? (yes/no/other)** | **Clinical Action/Change** | **Death (time to)** | **Cause of death if within 2mos?** | **Autopsy?** |
|---|---|---|---|---|---|
| 1 | Yes | Medication Change/Removal | alive | | |
| 2 | No | confirmed Medication Choice | =< 1 yr | n/a | No |
| 3 | No | confirmed Medication Choice | alive | | |
| 4 | N/A | no changes | alive | | |

| | | | | | |
|---|---|---|---|---|---|
| 5 | No | Medication Change/Removal | alive | | |
| 6 | N/A | No Changes | | | |
| 6 | Yes | Medication Change | alive | | |
| 6 | N/A | Medication Change | | | |
| 7 | No | Medication Change/Addition | =< 30 days | Fungal infection significant contribution | No |
| 8 | No | confirmed Medication Choice | =< 30 days | Underlying disease | Yes |
| 9 | No | Medication Change/Addition | =< 60 days | Fungal infection significant contribution | Yes |
| 10 | N/A | No Change | alive | | |
| 11 | Yes | Medication Change/Addition | alive | | |
| 12 | No | confirmed Medication Choice | =< 1 yr | n/a | Yes |
| 13 | No | Medication Change/Removal | alive | | |
| 14 | No | Medication Change/ | alive | | |
| 15 | N/A | Medication Change/Removal | alive | | |
| 16 | Yes | Medication Change/Removal | Any Death (>1yr) | n/a | |
| 17 | No | confirmed Medication Choice | alive | | |
| 18 | Yes | Medication Change/Addition | Any Death (>1yr) | n/a | |
| 19 | No | confirmed Medication Choice | alive | | |
| 20 | No | Medication Change/Removal | Any Death (>1yr) | n/a | |
| 21 | No | Medication Change/Removal | alive | | |
| 22 | No | Medication Change/Addition | alive | | |
| 23 | No | confirmed Medication Choice | =< 60 days | Fungal infection significant contribution | No |
| 24 | Yes | Confirmed Medication Choice | Any Death (>1yr) | n/a | |

| | | | | | |
|---|---|---|---|---|---|
| 25 | No | Medication Change/Addition | =< 1 yr | n/a | No; no physician death note; d/c to home hospice at 22 days |
| 26 | Yes | Medication Change/Addition | =< 1 yr | n/a | No; no physician death note; transitioned to comfort only |
| 27 | N/A (patient died prior to result) | other (see notes) | =< 30 days | Other infection significant contribution<br>Fungal infection significant contribution | Yes |
| 28 | N/A | No Change | alive | n/a | |
| 29 | N/A | Confirmed Medication Choice | alive | n/a | |
| 30 | No | Medication Change/Addition | Any Death (>1yr) | n/a | |
| 31 | N/A | Medication Change/Removal | alive | n/a | |
| 32 | Unable to determine | confirmed ampho & Vori | Any Death (>1yr) | n/a | |
| 33 | N/A | medication Change/Removal | alive | n/a | |
| 34 | No | Medication Change/Addition | =< 1 yr | n/a | No |
| 35 | N/A | | alive | n/a | |
| 36 | N/A | medication Change/Removal | =< 1 yr | Not documented/Unknown | No |
| 37 | No | Medication Change/Addition | alive | n/a | |
| 38 | Yes | confirmed Medication Choice | alive | n/a | |
| 39 | Yes* (supported interpretation of culture as bystander) | No antifungals given | alive | n/a | |
| 40 | Yes | Medication Change/addition | alive | n/a | No |

| | | | | | |
|---|---|---|---|---|---|
| 41 | N/A | no Change | =< 1 yr | Not documented/Unknown | No |
| 42 | N/A | no change | =< 1 yr | n/a | Yes |
| 43 | No | medication Change/Removal | Any Death (>1yr) | n/a | |
| 44 | No | medication Change/Addition | =< 30 days | Fungal infection significant contribution | No |
| 45 | No | Medication Change/addition | =< 30 days | Fungal infection significant contribution | Yes |
| 46 | N/A | n/a | Any Death (>1yr) | n/a | |
| 47 | First identified by *Aspergillus fumigatus*-specific PCR, ordered concurrently | Medication Change/Addition | alive | | |
| 48 | First identified by *Aspergillus fumigatus*-specific PCR, ordered concurrently | medication change (posa to vori) | =< 1 yr | n/a | No |
| 49 | N/A | No Change | alive | | |
| 50 | No | No Change | =< 30 days | Other infection significant contribution | No |
| 51 | First identified by mucormycete-specific PCR, ordered concurrently | Confirmed Medication Choice | =< 30 days | Fungal infection significant contribution | No |
| 52 | First identified by *Aspergillus fumigatus*-specific PCR, ordered concurrently | Medication Change/Addition | =< 30 days | Fungal infection significant contribution | No |