Superior Accuracy of *Aspergillus* Plasma Cell-Free DNA Polymerase Chain Reaction Over Serum Galactomannan for the Diagnosis of Invasive Aspergillosis

Jordan Mah,[1,2] Veronica Nicholas,[1] Ralph Tayyar,[3] Angel Moreno,[1] Kanagavel Murugesan,[1] Indre Budvytiene,[2] and Niaz Banaei[1,2,3]

[1]Division of Pathology, Stanford University School of Medicine, Stanford, California, USA; [2]Clinical Microbiology Laboratory, Stanford Health Care, Stanford, California, USA; and [3]Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California, USA

(See the Editorial Commentary by White and Donnelly on pages 1291–3.)

Background. Invasive aspergillosis (IA) in immunocompromised hosts carries high morbidity and mortality. Diagnosis is often delayed because definitive diagnosis requires invasive specimen collection, while noninvasive testing with galactomannan is moderately accurate. Plasma cell-free DNA polymerase chain reaction (cfDNA PCR) represents a novel testing modality for the noninvasive diagnosis of invasive fungal disease (IFD). We directly compared the performance of *Aspergillus* plasma cfDNA PCR with serum galactomannan for the diagnosis of IA during routine clinical practice.

Methods. We conducted a retrospective study of all patients with suspected IFD who had *Aspergillus* plasma cfDNA PCR testing at Stanford Health Care from 1 September 2020 to 30 October 2022. Patients were categorized into proven, probable, possible, and no IA based on the EORTC/MSG definitions. Primary outcomes included the clinical sensitivity and specificity for *Aspergillus* plasma cfDNA PCR and galactomannan.

Results. Overall, 238 unique patients with *Aspergillus* plasma cfDNA PCR test results, including 63 positives and 175 nonconsecutive negatives, were included in this study. The majority were immunosuppressed (89.9%) with 22.3% 30-day all-cause mortality. The overall sensitivity and specificity of *Aspergillus* plasma cfDNA PCR were 86.0% (37 of 43; 95% confidence interval [CI], 72.7–95.7) and 93.1% (121 of 130; 95% CI, 87.4–96.3), respectively. The sensitivity and specificity of serum galactomannan in hematologic malignancies/stem cell transplants were 67.9% (19 of 28; 95% CI, 49.3–82.1) and 89.8% (53 of 59; 95% CI, 79.5–95.3), respectively. The sensitivity of cfDNA PCR was 93.0% (40 of 43; 95% CI, 80.9–98.5) in patients with a new diagnosis of IA.

Conclusions. *Aspergillus* plasma cfDNA PCR represents a more sensitive alternative to serum galactomannan for noninvasive diagnosis of IA.

Keywords. invasive aspergillosis; immunocompromised hosts; galactomannan; cell-free DNA PCR; noninvasive diagnostic testing.

Invasive fungal disease (IFD) with *Aspergillus* species in immunocompromised hosts is associated with high morbidity and mortality [1]. Early diagnosis of IFD is critical for the timely initiation of appropriate antifungal therapy, which can optimize patient outcomes and survival [2–4]. Conventional diagnostics, including fungal culture, histopathology, and targeted fungal sequencing, are not routinely possible due to the need for invasive specimen collection, which is challenging in this vulnerable population who are often clinically unstable and thrombocytopenic [1, 3, 5, 6]. Thus, serum galactomannan, a noninvasive fungal biomarker, is routinely used to aid with

diagnosis of invasive aspergillosis (IA). However, galactomannan is only modestly accurate for diagnosing IA (sensitivity, 71.0%; specificity, 89.0%) and therefore cannot be used to confirm or rule out IA, with its optimal performance restricted to neutropenic populations [1, 5–7]. Noninvasive testing with *Aspergillus* polymerase chain reaction (PCR) on whole blood has also been used for the diagnosis of IA, but clinical performance has been modest with an overall sensitivity and specificity of 81.1% and 72.4%, respectively [8, 9].

Cell-free DNA (cfDNA) PCR represents a novel, alternative means for the noninvasive diagnosis of IA based on detection of circulating fungal DNA fragments in serum and plasma [5, 8, 10–12]. Previous studies that used unoptimized preanalytical approaches for cfDNA testing have primarily focused on *Aspergillus* serum cfDNA PCR [13, 14]. However, more recent studies have demonstrated that *Aspergillus* plasma cfDNA has superior sensitivity compared with serum due to loss of trapped cfDNA during clot formation [15–18]. Furthermore, the diagnostic value of *Aspergillus* plasma cfDNA PCR compared with

serum galactomannan remains unknown. Previously, we optimized the preanalytical aspects of microbial plasma cfDNA detection and developed a fungal plasma cfDNA PCR panel for etiologies of IFD including *Aspergillus* spp., the most common cause of invasive fungal infections in immunocompromised patients [19, 20]. In the current study, we sought to evaluate the performance characteristics of an optimized *Aspergillus* plasma cfDNA PCR for the diagnosis of IA in a real-world setting, comparing it head-to-head with serum galactomannan to discern its optimal usage in different patient populations. We hypothesized that the preanalytically optimized *Aspergillus* plasma cfDNA PCR would be more sensitive compared with serum galactomannan for diagnosis of IA.

METHODS

Study Design

We conducted a retrospective case-control study of all adult and pediatric patients with suspected IFD who underwent *Aspergillus* plasma cfDNA PCR testing at the Stanford Health Care Clinical Microbiology Laboratory, Stanford, California, from 1 September 2020 to 30 October 2022. All patients with positive PCR results were included. Patients with negative PCR results were randomly selected at a ratio of approximately 3:1 (negative-to-positive). Patients with multiple PCR results were classified by their first positive PCR episode. Consecutive positives were defined by 2 positive results within 7 days. For duplicate negative PCR results, only the patient's first negative result was included in the analysis. Electronic medical records were retrospectively reviewed to collect data on patient demographics, comorbidities, types of immunosuppression, imaging results, type of infection, microbiological results, treatment, and clinical outcomes. Patients were categorized into proven, probable, possible, and no IA based on the consensus definitions by the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) [6]. Primary outcomes included the clinical sensitivity and specificity for *Aspergillus* plasma cfDNA PCR and serum galactomannan. Sensitivities were calculated using proven and probable IA EORTC/MSGERC criteria as the true positive reference standard, while specificity was calculated using no IA and proven or probable IFD caused by another fungus as the true negative reference standard [6]. When calculating the sensitivity of *Aspergillus* plasma cfDNA PCR, probable IA that only met the EORTC/MSGERC definition by 2 consecutive cfDNA PCR results as the mycological criteria were excluded from analysis, but these cases were included when measuring the sensitivity of galactomannan. Sensitivities and specificities for both *Aspergillus* plasma PCR and serum galactomannan were also calculated for patient subpopulations, which is further described in the Supplementary Material. Secondary outcomes included the positive and negative predictive value (PPV,

NPV) at a prevalence of 5% and 20% based on reported ranges for various patient populations [22], positive and negative likelihood ratios (PLR, NLR), diagnostic odds ratios (DORs), and sensitivity in proven and probable IA requiring an invasive procedure. The study design is shown in Figure 1.

Aspergillus PCR

The *Aspergillus* plasma cfDNA PCR panel was comprised of singleplex and multiplex reactions as previously described [19, 20]. In brief, cfDNA was extracted using the Maxwell RSC ccfDNA plasma kit (Promega; Fitchburg, WI) from 4 mL of plasma, followed by singleplex reaction for *Aspergillus* species (*Aspergillus fumigatus/Aspergillus flavus/Aspergillus niger*) and a multiplex panel for *Aspergillus terreus* and *Aspergillus ustus/Aspergillus nidulans*. Separate reactions were included for external positive and negative controls as well as an internal control using the human beta-globin gene to assess for DNA extraction and PCR inhibition. For beta-globin, a cycle threshold (CT) value ≤40 was required for a negative result to be considered valid. A CT value ≤45 for *Aspergillus* PCR panel was considered positive. There were no restrictions to providers on who could order the test.

Serum Galactomannan

Serum galactomannan was performed using the Platella *Aspergillus* Ag assay (Bio-Rad; Hercules, CA) according to the manufacturer's instructions and reported as positive (≥0.5 optical density index [ODI]) or negative (<0.5 ODI) with the corresponding ODI value. Values >2.0 ODI were reported as ≥2.0 ODI.

Data Analyses

Continuous data were summarized as means with standard deviations (SDs) and compared using *t* tests using SPSS Version v28.0. The Fisher exact test was used to determine the significance of the differences between proportions. Sensitivity, specificity, PPV, NPV, PLR, and NLR were calculated using the MedCalc Diagnostic test evaluation calculator with 95% confidence intervals (CIs; https://www.medcalc.org/calc/diagnostic_test.php [21]).

Ethics

The Stanford University Institutional Review Board approved the study.

RESULTS

Patient Characteristics

Overall, 238 patients with suspected IFD and *Aspergillus* plasma cfDNA PCR test results were included in this study. They consisted of 63 consecutive positive PCR results and 175 randomly selected negative cfDNA PCR results. The mean age of the cohort was 50.7 years (SD ± 21.4), and 55.5% were male. The majority (89.9%) were immunosuppressed due to
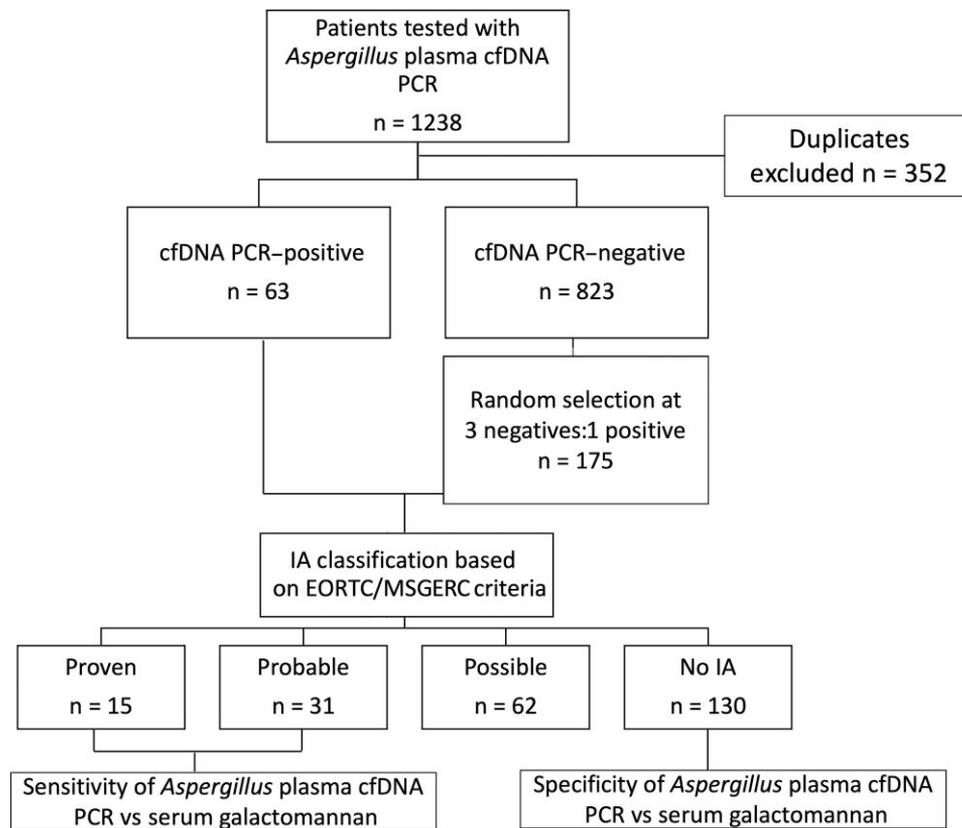
Figure 1. Study design. Abbreviations: cfDNA PCR, cell-free DNA polymerase chain reaction; EORTC/MSGERC, European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium; IA, invasive aspergillosis.

hematopoietic stem cell transplant (HSCT; 27.3%), hematological malignancy (HM; 26.5%), solid organ transplantation (SOT; 18.0%), solid organ malignancy (8.8%), or other causes (9.2%). Of the nonimmunosuppressed population, 33.3% had diabetes. Per EORTC/MSGERC criteria definitions for IA, 15 (6.3%) had proven, 31 (13.0%) had probable, 62 (26.1%) had possible, and 130 (54.6%) had no IFD caused by *Aspergillus*. Of the probable IA, criteria for mycological evidence were met based on positive serum galactomannan in 21 (67.7%), based on 2 consecutive positive PCR in 3 (9.7%), and by other mycologic criteria in 7 (22.6%). Of the 62 possible IA cases, 47 (77.0%) received treatment for IA. Among patients with no IA, 15.4% (20 of 130) had an IFD due to another fungus (14 Mucorales agents, 1 *Fusarium* spp., 4 *Scedosporium* spp./ *Lomentospora prolificans*, and 1 dematiaceous). In the entire cohort, the 30-day all-cause mortality was 22.3% (53 of 238), with 39.1% (93 of 238) receiving an invasive procedure and 39.5% (94 of 238) receiving mold prophylaxis (Table 1).

Accuracy of *Aspergillus* Plasma cfDNA PCR

In proven or probable IA, *Aspergillus* plasma cfDNA PCR had an overall sensitivity of 86.0% (37 of 43; 95% CI, 72.7–93.4; Table 2). When the 3 probable IA that met only the EORTC/

MSGERC mycological criteria due to 2 consecutive positive cfDNA PCR results were included, sensitivity was 87.0% (40 of 46; 95% CI, 73.7–95.0). In patients with 2 consecutive positive *Aspergillus* plasma cfDNA PCR results, the sensitivity was 90.9% (20 of 22; 95% CI, 70.8–98.9). When cases with a previous diagnosis of IA >14 days from the initial positive cfDNA PCR were excluded, the sensitivity of *Aspergillus* plasma cfDNA PCR was 93.0% (40 of 43; 95% CI, 80.9–98.5). The test sensitivities in various immunosuppressed populations on mold prophylaxis are shown in Table 2. In patients with no IA, *Aspergillus* plasma cfDNA PCR had an overall specificity of 93.1% (121 of 130; 95% CI, 87.4–96.3), with a similar performance in other populations (Table 2).

With prevalence set at 20.0%, *Aspergillus* PCR had an overall PPV of 75.7% (95% CI, 60.8–87.1) and NPV of 96.4% (95% CI, 91.5–98.9); at 5% prevalence, PCR had a PPV of 39.6% (95% CI, 25.5–55.0) and NPV of 99.2% (95% CI, 95.7–100.0; Table 3). For proven and probable IA, *Aspergillus* plasma cfDNA PCR had an overall positive and negative likelihood ratio of 12.43 and 0.15, respectively, with a DOR of 82.86. Results for subpopulations are shown in Table 3. A summary of invasive *Aspergillus* infection cases with negative *Aspergillus* plasma cfDNA PCR is provided in Table 4.

Table 1. Patient Characteristics

Characteristic	Overall (N = 238)	*Aspergillus* Plasma cfDNA PCR Positive (n = 63[a])	*Aspergillus* Plasma cfDNA PCR Negative (n = 175)
Mean age ± standard deviation, y	50.7 ± 21.4	52.8 ± 21.8	49.9 ± 21.3
Sex, no. (%)			
Male	132 (55.5)	39 (61.9)	93 (53.1)
Immunosuppression, no. (%)	214 (89.9)	61 (96.8)	153 (87.4)
Type of immunosuppression, no. (%)			
Hematopoietic stem cell transplant	65 (27.3)	18 (28.6)	47 (26.8)
Solid organ transplantation	43 (18.0)	8 (12.7)	35 (20.0)
Hematological malignancy	63 (26.5)	22 (34.9)	41 (23.4)
Solid organ malignancy	21 (8.8)	5 (7.9)	16 (9.1)
Other	22 (9.2)	8 (12.6)	14 (8.0)
None	24 (10.0)	2 (3.2)	22 (12.6)
Diagnosis of IA based on			
European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium criteria, no. (%)			
Proven	15 (6.3)	12 (19.0)	3 (1.7)
Probable	31 (13.0)	28 (44.4)	3 (1.7)
Possible	62 (26.1)	14 (22.2)	48 (27.6)
None	130 (54.6)	9 (14.3)	121 (69.5)
Clinical presentation, no. (%)			
Pulmonary	195 (81.9)	53 (84.1)	142 (81.1)
Disseminated	16 (6.7)	3 (4.8)	13 (7.4)
Localized	21 (8.8)	6 (9.5)	15 (8.6)
Unknown	6 (2.5)	1 (1.6)	5 (2.9)
Mold prophylaxis, no. (%)	94 (39.5)	23 (36.5)	71 (40.6)
Received an invasive procedure, no. (%)	93 (39.1)	26 (41.3)	67 (38.3)
30-day all-cause mortality,[b] no. (%)	53 (22.3)	23 (36.5)	30 (17.1)
Mycological evidence in probable IA, no. (%)			
2 consecutive PCR positives	3 (9.7)	N/A	N/A
Serum galactomannan[c]	21 (67.7)	N/A	N/A
Other mycological criteria[d]	7 (22.6)	N/A	N/A
Antifungals administered in possible IA, no. (%)			
Yes	48 (77.4)	N/A	N/A
No	14 (22.6)	N/A	N/A
No IFD, no. (%)			
No IA	110 (84.6)	N/A	N/A
Proven or probable Mucorales order	14 (10.8)	N/A	N/A
Proven or probable *Fusarium* spp.	1 (0.8)	N/A	N/A
Proven or probable *Scedosporium apiospermum/Lomentospora prolificans*	4 (3.1)	N/A	N/A
Proven dematiaceous mold	1 (0.8)	N/A	N/A

Abbreviation: cfDNA PCR, cell-free DNA polymerase chain reaction; IA, invasive aspergillosis; N/A, not applicable; IFD, invasive fungal disease.

[a]A total of 63 had at least 1 positive; 25 had a repeat test, of which 20 were positive and 5 were negative.

[b]From the time of *Aspergillus* plasma cfDNA PCR.

[c]Galactomannan >1.0 for single serum.

[d]Galactomannan >1.0 cerebrospinal fluid or bronchoalveolar lavage (BAL); *Aspergillus* species recovered in culture from sputum, BAL, bronchial brush, or aspirate.

Accuracy of Serum Galactomannan

In proven/probable IA, serum galactomannan had an overall sensitivity of 63.0% (29 of 46; 95% CI, 48.6–75.5). The sensitivity was 67.9% (19 of 28; 95% CI, 49.3–82.1) in HM/HSCT populations, 62.2% (23 of 37; 95% CI, 46.1–75.9) in HM/HSCT/SOT population, and 55.6% (5 of 9; 95% CI, 26.7–81.1) in HM/HSCT patients who were on mold prophylaxis (Table 2). In patients with no IA, serum galactomannan had an overall specificity of 93.0% (106 of 114; 95% CI, 86.8–96.9); in the HM/HSCT cohort, the specificity was 89.8% (53 of 59; 95% CI, 79.5–95.3), with similar performance in HM/HSCT/SOT populations (Table 2).

In patients with HM/HSCT and prevalence set at 20.0%, serum galactomannan had a PPV of 62.5% (95% CI, 41.1–80.9) and NPV of 91.8% (95% CI, 82.0–97.2; Table 3). When restricted to HM/HSCT patients, serum galactomannan had a PLR and NLR of 6.67 and 0.36, respectively, with a DOR of 18.53 (Table 3).

Table 2. Sensitivity and Specificity for *Aspergillus* Plasma Cell-Free DNA Polymerase Chain Reaction and Serum Galactomannan for the Diagnosis of Invasive *Aspergillus* Infection

% Sensitivity, 95% CI

		Aspergillus Plasma Cell-Free DNA Polymerase Chain Reaction				Serum Galactomannan[a]		
Definition of true positive		Overall	HM/HSCT	HM/HSCT/SOT	SOT/Other[b]	2 Consecutive Positives	Overall	HM/HSCT	HM/HSCT/SOT
	Proven IA	80.0 (12/15; 95% CI, 54.8–93.0)	85.7 (6/7; 95% CI, 48.7–97.4)	83.3 (10/12; 95% CI, 43.7–96.7)	71.4 (5/7; 95% CI 29.0–96.3)	71.4 (5/7; 95% CI, 29.0–96.3)	53.3 (8/15; 95% CI, 30.1–75.2)	71.4 (5/7; 95% CI, 35.9–91.8)	58.3 (7/12; 95% CI, 32.0–80.1)
	Probable IA	[c]89.3 (25/28; 95% CI, 72.8–96.3)[d]	[c]94.4 (17/18; 95% CI, 74.2–99.0)[d]	[c]86.4 (19/22; 95% CI, 66.7–95.3)	80.0 (8/10; 95% CI 44.4–97.5)	100 (15/15; 95% CI, 78.2–100)	70.0 (21/31; 95% CI, 50.1–81.4)	66.7 (14/21; 95% CI, 45.4–82.8)	64.0 (16/25; 95% CI, 44.5–79.8)
	Proven or probable IA	[c]86.0 (37/43; 95% CI, 72.7–93.4)[d]	[c]92.0 (23/25; 95% CI, 81.4–100)[d]	[c]85.3 (29/34; 95% CI, 69.9–93.6)	76.5 (13/17; 95% CI 50.1–93.2)	90.9 (20/22; 95% CI, 70.8–98.9)	63.0 (29/46; 95% CI, 48.6–75.5)	67.9 (19/28; 95% CI, 49.3–82.1)	62.2 (23/37; 95% CI, 46.1–75.9)
	Proven or probable IA on mold prophylaxis	[c]84.6 (11/13; 95% CI, 57.7–95.7)	[c]83.3 (5/6; 95% CI, 75.0–97.8)	[c]81.8 (9/11; 95% CI, 52.3–94.9)	85.7 (6/7; 95% CI 42.1–99.6)	100 (9/9; 95% CI, 66.4–100)	56.3 (9/16; 95% CI, 33.2–76.9)	55.6 (5/9; 95% CI, 26.7–81.1)	50.0 (7/14; 95% CI, 26.8–73.2)

% Specificity, 95% CI

		Aspergillus Plasma cfDNA PCR				Serum Galactomannan		
Definition of true negative		Overall	HM/HSCT	HM/HSCT/SOT	SOT/Other[b]	Overall	HM/HSCT	HM/HSCT/SOT
	No IA	93.1 (121/130; 95% CI, 87.4–96.3)	91.8 (56/61; 95% CI, 82.2–96.5)	94.0 (78/83; 95% CI, 86.7–97.4)	93.6 (44/47; 95% CI, 82.5–98.7)	93.0 (106/114; 95% CI, 86.8–96.9)	89.8 (53/59; 95% CI, 79.5–95.3)	92.2 (71/77; 95% CI, 84.0–96.4)
	No IA on mold prophylaxis	95.7 (45/47; 95% CI, 85.8–98.8)	96.7 (29/30; 95% CI, 83.3–99.4)	97.6 (41/42; 95% CI, 87.7–99.6)	93.8 (15/16; 95% CI, 69.8–99.8)	95.1 (39/41; 95% CI, 83.9–98.7)	92.9 (26/28; 95% CI, 77.4–98.0)	94.7 (36/38; 95% CI, 82.7–98.5)
	No IA and possible IA[e]	88.0 (169/192; 95% CI, 82.6–92.3)	86.0 (86/100; 95% CI, 77.9–91.5)	88.1 (118/134; 95% CI, 81.5–92.5)	88.4 (61/69; 95% CI, 78.4–94.9)	94.1 (160/170; 95% CI, 89.5–97.1)	93.7 (89/95; 95% CI, 86.9–97.1)	93.5 (115/123; 95% CI, 87.7–96.7)

Abbreviations: cfDNA PCR, cell-free DNA polymerase chain reaction; CI, confidence interval; HM, hematological malignancy; HSCT, hematopoietic stem cell transplant; IA, invasive aspergillosis; SOT, solid organ transplantation.

[a]Serum galactomannan results displayed only for populations that have evidence (HM/HSCT/SOT).

[b]"Other" refers to immunosuppression that did not include HM/HSCT/SOT.

[c]Probable IA that was met criteria by 2 consecutive positive PCR assays as the mycological criteria were excluded from calculation for *Aspergillus* plasma cfDNA PCR.

[d]Refers to statistical significance between the corresponding comparator group for serum galactomannan.

[e]Includes 14 cases of possible IA that had a single positive *Aspergillus* plasma cfDNA PCR result.

Table 3. Performance Characteristics of *Aspergillus* Plasma Cell-Free DNA Polymerase Chain Reaction and Serum Galactomannan for the Diagnosis of Invasive *Aspergillus* Infections

	Aspergillus Plasma Cell-Free DNA Polymerase Chain Reaction				Serum Galactomannan[a]		
	Overall	HM/HSCT	HM/HSCT/SOT	SOT/Other[b]	Overall	HM/HSCT	HM/HSCT/SOT
Positive predictive value[c]							
Prevalence 5%	39.6 (25.5–55.0)	37.1 (19.8–57.3)	42.7 (26.0–60.8)	38.7 (16.0–65.6)	32.1 (17.8–49.5)	26.0 (10.7–47.3)	29.6 (14.2–49.3)
Prevalence 20%	75.7 (60.8–87.1)	73.7 (53.8–88.4)	78.0 (60.5–90.3)	75.0 (47.6–92.7)	69.2 (51.9–96.9)	62.5 (41.1–80.9)	66.6 (46.8–82.9)
Negative predictive value[c]							
Prevalence 5%	99.2 (95.7–100.0)	99.5 (93.0–100.0)	99.2 (94.1–100.0)	98.7 (90.2–100.0)	98.0 (93.6–99.7)	98.2 (91.0–99.9)	97.9 (92.1–99.8)
Prevalence 20%	96.4 (91.5–98.9)	97.9 (90.1–99.9)	96.2 (89.6–99.2)	94.1 (83.3–98.8)	91.0 (84.5–95.4)	91.8 (82.0–97.2)	90.7 (82.4–95.9)
Positive LR (sensitivity/ 1 – specificity)	12.43	11.22	14.16	11.98	8.98	6.67	7.98
Negative LR (1 – Sensitivity/ Specificity)	0.15	0.09	0.16	0.25	0.40	0.36	0.41
Diagnostic odds ratio (positive LR/negative LR)	82.86	124.66	88.5	47.92	22.45	18.53	19.46

Abbreviations: HM, hematological malignancy; HSCT, hematopoietic stem cell transplant; LR, likelihood ratio; SOT, solid organ transplantation.

[a]Serum galactomannan results displayed only for populations that have evidence (HM/HSCT/SOT).

[b]"Other" refers to immunosuppression that did not include HM/HSCT/SOT.

[c]Reported as percentage with 95% confidence intervals.

Plasma cfDNA PCR vs Serum Galactomannan

In proven/probable IA in HM/HSCT populations, the sensitivity of *Aspergillus* plasma cfDNA PCR was higher than that of serum galactomannan (92.0%; 95% CI, 81.4–100 vs 67.9%; 95% CI, 49.3–82.1; *P* = .043), but the specificities were similar (91.8%; 95% CI, 82.2–96.5 vs 89.8%; 95% CI, 79.5–95.3; *P* = .761; Supplementary Table 1). The sensitivity of *Aspergillus* plasma cfDNA PCR was higher than the sensitivity of serum galactomannan in patients who underwent an invasive procedure (81.0%; 17 of 21; 95% CI, 60.0–92.3 vs 47.6%; 10 of 21; 95% CI, 28.3–67.6; *P* = .024).

DISCUSSION

Diagnosis of IFD using circulating fungal plasma cfDNA PCR is a novel noninvasive diagnostic approach that holds great potential for shortening the time to diagnosis and improving outcomes. However, much remains to be learned about its diagnostic accuracy and prognostic value compared with existing noninvasive diagnostics [23, 24]. In this study, we evaluated the clinical accuracy of a preanalytically optimized *Aspergillus* plasma cfDNA PCR assay while in use to diagnose immunocompromised patients with suspected IFD [19, 20]. We also directly compared the performance of *Aspergillus* plasma cfDNA PCR with serum galactomannan for the noninvasive diagnosis of IA.

We found that *Aspergillus* plasma cfDNA PCR is highly sensitive (93.0%) and specific (93.1%) for a new diagnosis of IA. Compared with the few published studies that performed *Aspergillus* cfDNA PCR on plasma [15, 17], the sensitivity of our assay was comparable (93.0% vs 90.9%–94.7%), but the specificity was higher (93.1% vs 52.8%–83.3%). Compared with a recent metanalysis that included all studies that used whole blood and serum, the assay in the current study was both more sensitive (93.0% vs 71.0%–85.5%) and specific (93.1% vs 69.9%–86.6%) [9, 18]. The superior sensitivity observed in our study may be related to optimization of preanalytical factors such as specimen type (ie, plasma), sample volume, and extraction method [19, 20, 25]. Previous studies have demonstrated that large-volume extraction of plasma provides the highest sensitivity and that plasma is superior to serum because it mitigates the loss of trapped cfDNA during clot formation [17–19, 25, 26]. Another possible explanation for the higher accuracy reported in our study may be related to how *Aspergillus* cfDNA PCR was used at our institution for diagnosis of IA in symptomatic patients with radiologic evidence of IFD (ie, pulmonary lesions); in the majority of previous studies, it has been used to screen asymptomatic patients [9, 10, 12]. Thus, the stage of IA could have impacted the sensitivity reported in the current study.

Of the 6 cases of proven/probable IA that tested negative with *Aspergillus* plasma cfDNA PCR in this study, half had a prior diagnosis of IA and were already on antifungal therapy, while the other half had received empiric antimold therapy prior to testing. All other cases of IA that had a positive *Aspergillus* plasma cfDNA PCR had a new diagnosis of IA, suggesting that the utility of *Aspergillus* plasma cfDNA PCR is greatest in patients with a new diagnosis of IA. The relative timing of PCR testing with respect to therapy is important as sensitivity has been shown to diminish after initiation of therapy [27], which is consistent with our observation that PCR rapidly reverts to negative within 2 weeks in the majority of IA patients on appropriate antifungal therapy (authors' unpublished data).

Table 4. Summary of Clinical and Laboratory Findings for Invasive *Aspergillus* Infection Cases With Negative *Aspergillus* Plasma Cell-Free DNA Polymerase Chain Reaction

Clinical History	Classification by the European Organization for Research and Treatment of Cancer Criteria and Mycoses Study Group Education and Research Consortium	Type of Infection	Indication for *Aspergillus* Plasma cfDNA PCR	Relative Timing of *Aspergillus* Plasma cfDNA PCR	Serum Galactomannan Positivity	Antifungal Prophylaxis	On Antifungal Treatment Prior to *Aspergillus* Plasma cfDNA PCR
53-year-old male with recent liver transplant for decompensated cirrhosis; presents with worsening respiratory status	Probable IA	Pulmonary aspergillosis	Diagnosis	Same day of diagnosis with positive galactomannan >2.0 OD	Yes	Yes, caspofungin for *Candida* prophylaxis 7 days prior to *Aspergillus* plasma cfDNA PCR	No
35-year-old male status post stem cell transplant for relapsed acute lymphoblastic leukemia with liver and skin graft vs host disease (grade 4); presents with worsening respiratory status with cavitation and consolidation	Probable IA	Pulmonary aspergillosis	Diagnosis	2 days prior to positive galactomannan 1.32 OD	Yes	Yes, previously on Posaconazole prophylaxis for past 24 weeks and 3 days prior to *Aspergillus* plasma cfDNA PCR	Yes, had received 3 days of liposomal amphotericin B.
56-year-old female with bilateral lung transplant for pulmonary sarcoidosis and with worsening respiratory status due to proven invasive aspergillosis	Proven IA	Pulmonary aspergillosis	Follow-up testing	21 days after histopathological diagnosis	No	Yes, previously on itraconazole for past 25 weeks prior to *Aspergillus* plasma cfDNA PCR	Yes, had received 21 days of isuvaconazole
54-year-old male with pure red cell aplasia status post recent stem cell transplant; presents with neutropenic fever with right middle lobe infiltrate	Proven IA	Pulmonary aspergillosis	Diagnosis	Same day of diagnosis with positive galactomannan >2.0 OD	Yes	No	Yes, had received 4 days of liposomal amphotericin B and isuvaconazole
57-year-old male with bilateral lung transplant for coronavirus disease 2019 acute respiratory distress syndrome and diagnosis of aspergillosis; presents with worsening respiratory status	Probable IA	Pulmonary aspergillosis	Follow-up testing	2 months after initial diagnosis	No	No	Yes, had received posaconazole for 2 months
35-year-old male with squamous cell carcinoma and sarcoidosis on capecitabine/nivolumab; known history of mandibular *Aspergillus* osteomyelitis; presents with worsening symptoms	Proven IA	Aspergillosis osteomyelitis	Follow-up testing	9 months after histopathological diagnosis	No	No	Yes had received voriconazole for the past 9 months

Abbreviations: cfDNA PCR, cell-free DNA polymerase chain reaction; IA, invasive aspergillosis; OD, optical density.

Similar to other studies of *Aspergillus* PCR on blood [9, 28], the sensitivity and specificity of *Aspergillus* plasma cfDNA PCR in the current study were highest in HM/HSCT patients. The accuracy was modest in other immunosuppressed populations, including SOT and other types of immunosuppression (sensitivity, 76.5%; specificity, 92.6%), which is inconsistent with prior studies that have shown poor sensitivity in other immunosuppressed populations, ranging from 0% to 62.5% [29–33]. Our findings therefore support the use of *Aspergillus* plasma cfDNA PCR in nonneutropenic patients, which is contrary to what is recommended by previous studies [29–33]. Additionally, we found that *Aspergillus* PCR remained sensitive in patients on mold prophylaxis, with a sensitivity ranging from 81.8% to 85.7% depending on the specific patient population and sensitivity of 90.9% in those with 2 consecutive positive PCRs, suggesting that PCR can be used to diagnose breakthrough infections. Previous studies have discouraged use of *Aspergillus* PCR in patients on mold prophylaxis due to diminished specificity of PCR in this population; however, we did not find a significant change in specificity between these 2 groups [30, 33–35]. This discrepancy may be explained by the fact that, unlike in the current study, previous studies used *Aspergillus* PCR to screen asymptomatic patients [9, 10, 12, 35–40].

The availability of highly accurate *Aspergillus* plasma cfDNA PCR requires a better understanding of how it compares with or complements existing noninvasive fungal biomarkers such as *Aspergillus* galactomannan. A unique feature of the current study is that it is the first to directly compare *Aspergillus* plasma cfDNA PCR with serum galactomannan. We demonstrate that *Aspergillus* plasma cfDNA PCR has a higher clinical sensitivity but similar specificity compared with serum galactomannan for the diagnosis of IA in a diverse population of immunosuppressed patients as well as those on mold prophylaxis. Although serum has been shown to be inferior to plasma for cfDNA testing, a meta-analysis comparing the performance of *Aspergillus* serum cfDNA PCR with serum galactomannan demonstrated that these tests are equivalent in their diagnostic accuracy [13]. However, of the studies included, none had directly compared these 2 assays head-to-head within the same study. The modest overall sensitivity (67.9%) and specificity (89.8%) of serum galactomannan in HM/HSCT patients in the current study are consistent with previously reported performance in this population (sensitivity, 58%–65%; specificity, 65%–95%) [7]. When comparing *Aspergillus* plasma cfDNA PCR with serum galactomannan across respective patient populations, the accuracy of cfDNA PCR is superior. Given the higher sensitivity of *Aspergillus* plasma cfDNA PCR compared with serum galactomannan, there is an opportunity to improve accurate and timely diagnosis of IA with the use of plasma cfDNA PCR. However, in some patients, serum galactomannan may still complement plasma cfDNA PCR. This was the case for 3 of 6 proven/probable IA patients who were negative by

Aspergillus plasma cfDNA PCR but had a positive galactomannan. Combining cfDNA PCR with serum galactomannan is a strategy that has been used previously to improve the diagnosis of IA, with an overall combined sensitivity ranging from 73% to 100% compared with 56% to 71.7% for *Aspergillus* serum PCR alone [32, 41–45]. In the current study, when *Aspergillus* plasma cfDNA PCR and serum galactomannan were combined, the overall sensitivity increased from 86.0% to 93.5% in all patients and to 100% in patients with new diagnoses of IA.

Although the findings of this study are encouraging, some limitations must be considered. First, this was a retrospective study at a single center; therefore, the generalizability of the findings may be limited. Further studies are needed to show whether our findings are reproducible at other centers with different patient populations, incidence of IAs, and test utilization and regional practices. Second, the study was designed to randomly include a portion (20%; 175 of 886) of suspected IFD patients with negative *Aspergillus* plasma cfDNA PCR results. If PCR-negative IA cases were missed with this design, the rate of such events must be extremely low and therefore unlikely to change the study findings. Third, the EORTC/MSGERC definition of probable IA could have introduced bias in the study findings. We avoided overestimation of *Aspergillus* plasma cfDNA PCR by excluding cases that met probable definition based only on 2 consecutive *Aspergillus* PCRs from plasma. However, by using serum galactomannan as part of the mycological criterion for probable IA, we likely overestimated the sensitivity of galactomannan as there were IA patients with negative serum galactomannan who were clinically diagnosed by PCR alone. This speaks to the inherent difficulty of using the EORTC/MSGERC IFD definitions in diagnostic accuracy studies, as the performance of a novel test may be underestimated by an imperfect reference standard. If we defined the reference standard to include patients with a single positive PCR as possible IA cases, the overall sensitivity of serum galactomannan is even lower at 48.3%.

In summary, *Aspergillus* plasma cfDNA PCR is a novel noninvasive diagnostic modality with improved sensitivity over serum galactomannan for the diagnosis of IA in a diverse patient population that may further improve the timely diagnosis and outcome of patients with IA.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

appraisal of manuscript drafts. All authors critically revised the manuscript for important intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Terrero-Salcedo D, Powers-Fletcher MV. Updates in laboratory diagnostics for invasive fungal infections. J Clin Microbiol 2020; 58:e01487-19.
2. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2008; 46:327–60.
3. Patterson TF, Thompson GR, Denning DW, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 63:e1–60.
4. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood 2002; 100:4358–66.
5. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. Lancet Infect Dis 2005; 5:609–22.
6. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis 2020; 71:1367–76.
7. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006; 42:1417–27.
8. White PL, Bretagne S, Klingspor L, et al. *Aspergillus* PCR: one step closer to standardization. J Clin Microbiol 2010; 48:1231–40.
9. Cruciani M, Mengoli C, Barnes R, et al. Polymerase chain reaction blood tests for the diagnosis of invasive aspergillosis in immunocompromised people. Cochrane Database Syst Rev 2019; 9:CD009551.
10. Arvanitis M, Ziakas PD, Zacharioudakis IM, Zervou FN, Caliendo AM, Mylonakis E. PCR in diagnosis of invasive aspergillosis: a meta-analysis of diagnostic performance. J Clin Microbiol 2014; 52:3731–42.
11. Donnelly JP. Polymerase chain reaction for diagnosing invasive aspergillosis: getting closer but still a ways to go. Clin Infect Dis 2006; 42:487–9.
12. Mengoli C, Cruciani M, Barnes RA, Loeffler J, Donnelly JP. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 2009; 9:89–96.
13. White PL, Wingard JR, Bretagne S, et al. *Aspergillus* polymerase chain reaction: systematic review of evidence for clinical use in comparison with antigen testing. Clin Infect Dis 2015; 61:1293–303.
14. Mercier T, Castagnola E, Marr KA, Wheat LJ, Verweij PE, Maertens JA. Defining galactomannan positivity in the updated EORTC/MSGERC consensus definitions of invasive fungal diseases. Clin Infect Dis 2021; 72:S89–94.
15. Springer J, White PL, Hamilton S, et al. Comparison of performance characteristics of *Aspergillus* PCR in testing a range of blood-based samples in accordance with international methodological recommendations. J Clin Microbiol 2016; 54:705–11.
16. Loeffler J, Mengoli C, Springer J, et al. Analytical comparison of in vitro-spiked human serum and plasma for PCR-based detection of *Aspergillus fumigatus* DNA: a study by the European *Aspergillus* PCR Initiative. J Clin Microbiol 2015; 53:2838–45.
17. White PL, Barnes RA, Springer J, et al. Clinical performance of *Aspergillus* PCR for testing serum and plasma: a study by the European *Aspergillus* PCR Initiative. J Clin Microbiol 2015; 53:2832–7.
18. Barnes RA, White PL, Morton CO, et al. Diagnosis of aspergillosis by PCR: clinical considerations and technical tips. Med Mycol 2018; 56:S60–72.
19. Murugesan K, Hogan CA, Palmer Z, et al. Investigation of preanalytical variables impacting pathogen cell-free DNA in blood and urine. J Clin Microbiol 2019; 57: e00782-19.
20. Senchyna F, Hogan CA, Murugesan K, et al. Clinical accuracy and impact of plasma cell-free DNA fungal polymerase chain reaction panel for noninvasive diagnosis of fungal infection. Clin Infect Dis 2021; 73:1677–84.
21. MedCalc Statistical Software version 19.2.6. Ostend, Belgium: MedCalc Software bv, 2020. https://www.medcalc.org
22. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi (Basel) 2017; 3:57.
23. Millon L, Caillot D, Berceanu A, et al. Evaluation of serum Mucorales polymerase chain reaction (PCR) for the diagnosis of mucormycoses: the MODIMUCOR prospective trial. Clin Infect Dis 2022; 75:777–85.
24. Moreno A, Epstein D, Budvytiene I, Banaei N. Accuracy of *Pneumocystis jirovecii* plasma cell-free DNA PCR for noninvasive diagnosis of *Pneumocystis* pneumonia. J Clin Microbiol 2022; 60:e0010122.
25. Cook L, Starr K, Boonyaratanakornkit J, Hayden R, Sam SS, Caliendo AM. Does size matter? Comparison of extraction yields for different-sized DNA fragments by seven different routine and four new circulating cell-free extraction methods. J Clin Microbiol 2018; 56:e01061-18.
26. Springer J, Schloßnagel H, Heinz W, et al. A novel extraction method combining plasma with a whole-blood fraction shows excellent sensitivity and reproducibility for patients at high risk for invasive aspergillosis. J Clin Microbiol 2012; 50: 2585–91.
27. Hummel M, Spiess B, Cornely OA, Dittmer M, Mörz H, Buchheidt D. *Aspergillus* PCR testing: results from a prospective PCR study within the AmBiLoad trial. Eur J Haematol 2010; 85:164–9.
28. Springer J, Morton O, Perry M, et al. Multicenter comparison of serum and whole-blood specimens for detection of *Aspergillus* DNA in high-risk hematological patients. J Clin Microbiol 2013; 51:1445.
29. Boch T, Reinwald M, Spiess B, et al. Detection of invasive pulmonary aspergillosis in critically ill patients by combined use of conventional culture, galactomannan, 1–3-beta-D-glucan and *Aspergillus* specific nested polymerase chain reaction in a prospective pilot study. J Crit Care 2018; 47:198–203.
30. Egger M, Jenks JD, Hoenigl M, Prattes J. Blood *Aspergillus* PCR: the good, the bad, and the ugly. J Fungi 2020; 6:18.
31. Blennow O, Remberger M, Klingspor L, et al. Randomized PCR-based therapy and risk factors for invasive fungal infection following reduced-intensity conditioning and hematopoietic SCT. Bone Marrow Transplant 2010; 45:1710–8.
32. Imbert S, Gauthier L, Joly I, et al. *Aspergillus* PCR in serum for the diagnosis, follow-up and prognosis of invasive aspergillosis in neutropenic and nonneutropenic patients. Clin Microbiol Infect 2016; 22:562.e1–8.
33. Springer J, Lackner M, Nachbaur D, et al. Prospective multicentre PCR-based *Aspergillus* DNA screening in high-risk patients with and without primary antifungal mould prophylaxis. Clin Microbiol Infect 2016; 22:80–6.
34. Jenks JD, Salzer HJF, Hoenigl M. Improving the rates of *Aspergillus* detection: an update on current diagnostic strategies. Expert Rev Anti Infect Ther 2019; 17:39–50.
35. Cruciani M, White PL, Mengoli C, et al. The impact of anti-mould prophylaxis on *Aspergillus* PCR blood testing for the diagnosis of invasive aspergillosis. J Antimicrob Chemother 2021; 76:635–8.
36. Florent M, Katsahian S, Vekhoff A, et al. Prospective evaluation of a polymerase chain reaction-ELISA targeted to *Aspergillus fumigatus* and *Aspergillus flavus* for the early diagnosis of invasive aspergillosis in patients with hematological malignancies. J Infect Dis 2006; 193:741–7.
37. Halliday C, Hoile R, Sorrell T, et al. Role of prospective screening of blood for invasive aspergillosis by polymerase chain reaction in febrile neutropenic recipients of haematopoietic stem cell transplants and patients with acute leukaemia. Br J Haematol 2006; 132:478–86.
38. Jordanides NE, Allan EK, McLintock LA, et al. A prospective study of real-time panfungal PCR for the early diagnosis of invasive fungal infection in haemato-oncology patients. Bone Marrow Transplant 2005; 35:389–95.
39. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-β-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. J Clin Microbiol 2004; 42:2733–41.
40. Suarez F, Lortholary O, Buland S, et al. Detection of circulating *Aspergillus fumigatus* DNA by real-time PCR assay of large serum volumes improves early diagnosis of invasive aspergillosis in high-risk adult patients under hematologic surveillance. J Clin Microbiol 2008; 46:3772–7.
41. Aguado JM, Vazquez L, Fernandez-Ruiz M, et al. Serum galactomannan versus a combination of galactomannan and polymerase chain reaction-based *Aspergillus* DNA detection for early therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled trial. Clin Infect Dis 2015; 60:405–14.
42. Bellanger AP, Millon L, Berceanu A, et al. Combining *Aspergillus* mitochondrial and ribosomal QPCR, in addition to galactomannan assay, for early diagnosis of invasive aspergillosis in hematology patients. Med Mycol 2015; 53:760–4.
43. Rogers TR, Morton CO, Springer J, et al. Combined real-time PCR and galactomannan surveillance improves diagnosis of invasive aspergillosis in high risk patients with haematological malignancies. Br J Haematol 2013; 161:517–24.
44. White PL, Parr C, Thornton C, Barnes RA. Evaluation of real-time PCR, galactomannan enzyme-linked immunosorbent assay (ELISA), and a novel lateral-flow device for diagnosis of invasive aspergillosis. J Clin Microbiol 2013; 51:1510–6.
45. Loeffler J, Hafner J, Mengoli C, et al. Prospective biomarker screening for diagnosis of invasive aspergillosis in high-risk pediatric patients. J Clin Microbiol 2017; 55:101–9.

Dovato
dolutegravir/lamivudine

EVIDENCE SUPPORTS THE HIGH BARRIER TO RESISTANCE OF DOVATO UP TO 5 YEARS[1-3]

CONFIDENCE IN DOVATO ACROSS TREATMENT SETTINGS[4-9]

Treatment-naïve resistance rates, with up to **3 years** of evidence[5-7]

0%
(n=0/1,885)*[,4]
REAL-WORLD EVIDENCE

0.1%
(n=1/953)**[,†,‡,§,5-7]
RANDOMISED CONTROLLED TRIALS

Treatment-experienced resistance rates, with up to **5 years** of evidence[1-3]

0.03%
(n=10/35,888)*[,4]
REAL-WORLD EVIDENCE

0%
(n=0/615)[||,¶,#,8,9]
RANDOMISED CONTROLLED TRIALS

>300,000 PEOPLE LIVING WITH HIV HAVE BEEN TREATED WITH DOVATO GLOBALLY[10]

DOVATO is supported by a wealth of evidence, with the outcomes of **>40,000** people living with HIV captured within clinical trials and real-world evidence, including those with:[4-9,11,12]

NO PRIOR TREATMENT EXPERIENCE[13]

NO BASELINE RESISTANCE TESTING[13]

HIGH BASELINE VIRAL LOAD (>100,000 copies/mL and even >1M copies/mL)[6,13]

LOW CD4 + COUNT (≤200 cells/mm^3)[13]

■ Patients from **phase III RCTs**
■ Patients from **unique real-world cohorts**

Year	Value
2015	>100
2016	>500
2017	>900
2018	>2,300
2019	>4,100
2020	>6,600
2021	>14,000
2022	>34,000
2023	>40,000

Year

IS IT TIME TO RECONSIDER THE VALUE OF THE 2ND NRTI?

LEARN MORE ⊙

DOVATO is indicated for the treatment of Human Immunodeficiency Virus type 1 (HIV-1) infection in adults and adolescents above 12 years of age weighing at least 40 kg, with no known or suspected resistance to the integrase inhibitor class, or lamivudine.[13]

Adverse events should be reported. Reporting forms and information can be found at **https://yellowcard.mhra.gov.uk/** or search for MHRA Yellowcard in the Google Play or Apple App store. Adverse events should also be reported to GSK on 0800 221441

REFERENCES

1. Maggiolo F et al. BMC Infect Dis 2022; 22(1): 782.
2. Taramasso L et al. AIDS Patient Care STDS 2021; 35(9): 342–353.
3. Ciccullo A et al. JAIDS 2021; 88(3): 234–237.
4. ViiV Healthcare. Data on File. REF-223795. 2024.
5. Cahn P et al. AIDS 2022; 36(1): 39–48.
6. Rolle C et al. Open Forum Infect Dis 2023; 10(3): ofad101.
7. Cordova E et al. Poster presented at 12th IAS Conference on HIV Science. 23–26 July 2023. Brisbane, Australia. TUPEB02.
8. De Wit S et al. Slides presented at HIV Glasgow. 23–26 October 2022. Virtual and Glasgow, UK. M041.
9. Llibre J et al. Clin Infect Dis 2023; 76(4): 720–729.
10. ViiV Healthcare. Data on File. REF-220949. 2024.
11. Rolle C et al. Poster presented IDWeek. 11–15 October 2023. Virtual and Boston, USA. 1603.
12. Slim J et al. Abstract presented IDWeek. 11–15 October 2023. Virtual and Boston, USA. 1593.
13. DOVATO. Summary of Product Characteristics. June 2023.

PRESCRIBING INFORMATION

Dovato Prescribing Information

Legal Notices

Privacy Policy

Contact Us

ViiV Healthcare, 980 Great West Road, Brentford, Middlesex, London, UK.
ViiV trademarks are owned by or licensed to the ViiV Healthcare group of companies.
Non-ViiV trademarks are owned by or licensed to their respective owners or licensors.
©2024 ViiV Healthcare group of companies or its licensor. All rights reserved.
Intended for healthcare professionals only.

ABBREVIATIONS

3TC, lamivudine; **CD4,** cluster of differentiation 4; **DTG,** dolutegravir; **FDA,** United States Food and Drug Administration; **FTC,** emtricitabine; **HIV,** human immunodeficiency virus; **ITT-E,** intention-to-treat exposed; **NRTI,** nucleoside/nucleotide reverse transcriptase inhibitor; **RCT,** randomised controlled trial; **RNA,** ribonucleic acid; **TAF,** tenofovir alafenamide fumarate; **TDF,** tenofovir disoproxil fumarate; **XTC,** emtricitabine.

FOOTNOTES

*Data extracted from a systematic literature review of DTG+3TC real-world evidence. Overlap between cohorts cannot be fully excluded.

**†The reported rate reflects the sum-total of resistance cases calculated from GEMINI I and II (n=1/716, through 144 weeks), STAT (n=0/131, through 52 weeks), and D2ARLING (n=0/106, through 24 weeks).[5-7]

†GEMINI I and II are two identical 148-week, phase III, randomised, double-blind, multicentre, parallel-group, non-inferiority, controlled clinical trials testing the efficacy of DTG/3TC in treatment-naïve patients. Participants with screening HIV-1 RNA ≤500,000 copies/mL were randomised 1:1 to once-daily DTG/3TC (n=716, pooled) or DTG + TDF/FTC (n=717, pooled). The primary endpoint of each GEMINI study was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 48 (ITT-E population, snapshot algorithm).[13]

‡STAT is a phase IIIb, open-label, 48-week, single-arm pilot study evaluating the feasibility, efficacy, and safety of DTG/3TC in 131 newly diagnosed HIV-1 infected adults as a first line regimen. The primary endpoint was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 24.[6]

§D2ARLING is a randomised, open-label, phase IV study designed to assess the efficacy and safety of DTG/3TC in treatment-naïve people with HIV with no available baseline HIV-1 resistance testing. Participants were randomised in a 1:1 ratio to receive DTG/3TC (n=106) or DTG + TDF/XTC (n=108). The primary endpoint was the proportion of participants with plasma HIV-1 RNA <50 copies/mL at Week 48.[7] Results at week 24 of the study.

||The reported rate reflects the sum-total of resistance cases calculated from TANGO (n=0/369, through 196 weeks) and SALSA (n=0/246, through 48 weeks).[8,9]

¶TANGO is a randomised, open-label, trial testing the efficacy of DOVATO in virologically suppressed patients. Participants were randomised in a 1:1 ratio to receive DOVATO (n=369) or continue with TAF-containing regimens (n=372) for up to 200 weeks. At Week 148, 298 of those on TAF-based regimens switched to DOVATO. The primary efficacy endpoint was the proportion of subjects with plasma HIV-1 RNA ≥50 copies/mL (virologic non-response) as per the FDA Snapshot category at Week 48 (adjusted for randomisation stratification factor).[8,13]

#SALSA is a phase III, randomised, open-label, non-inferiority clinical trial evaluating the efficacy and safety of switching to DTG/3TC compared with continuing current antiretroviral regimens in virologically suppressed adults with HIV. Eligible participants were randomised 1:1 to switch to once-daily DTG/3TC (n=246) or continue current antiretroviral regimens (n=247). The primary endpoint was the proportion of subjects with plasma HIV-1 RNA ≥50 copies/mL at Week 48 (ITT-E population, snapshot algorithm).[9]