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Dihydropteroate Synthase Mutations in *Pneumocystis* Pneumonia: Impact of Applying Different Definitions of Prophylaxis, Mortality Endpoints and Mutant in a Single Cohort

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Abstract

Pneumocystis jirovecii dihydropteroate synthase (DHPS) gene mutations are well-reported. Although sulfa prophylaxis generally is associated with DHPS mutant infection, whether mutant infection is associated with poorer clinical outcomes is less clear. The differing definitions of sulfa prophylaxis and the different mortality endpoints used in these studies may be one explanation for the conflicting study results. Applying different definitions of prophylaxis, mortality endpoints and DHPS mutant to 301 HIV-infected patients with *Pneumocystis* pneumonia, we demonstrate that prophylaxis, irrespective of definition, increased the risk of infection with pure mutant (any prophylaxis: AOR 4.00, 95% CI: 1.83–8.76, p<0.001) but not mixed genotypes (any prophylaxis: AOR 0.78, 95% CI: 0.26–2.36, p=0.65). However, infection with mutant DHPS, irrespective of definition, was not associated with increased mortality (all-cause or PCP death) at the three time-intervals examined (all p>0.05). Future studies should standardize key variables associated with DHPS mutant infection as well as examine DHPS mutant subtypes (pure mutant vs. mixed infections) – perhaps even individual DHPS mutant genotypes – so that data can be pooled to better address this issue.

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Keywords

Pneumocystis jirovecii; PCP; dihydropteroate synthase; DHPS; DHPS mutant

Introduction

Pneumocystis pneumonia (PCP) is a major cause of morbidity and mortality in HIV-infected individuals [1]. Although the use of sulfa drugs, trimethoprim-sulfamethoxazole (TMP-SMX) and dapsone, for prophylaxis has greatly decreased the incidence of PCP, sulfa prophylaxis has also been associated with mutations within the dihydropteroate synthase (DHPS) locus of *P. jirovecii* [2]. Sulfa drugs inhibit DHPS enzyme activity and because DHPS mutations are the mechanism of sulfa resistance in other organisms such as *Escherichia coli*, *Streptococcus pneumoniae* and *Plasmodium falciparum*, the presence of *Pneumocystis jirovecii* DHPS mutations suggests the emergence of sulfa resistance in this organism [3–5]. The association between these DHPS mutations and *P. jirovecii* resistance to sulfa drugs is supported by *in vitro* studies wherein insertion of mutations into the *Saccharomyces cerevisiae* DHPS that were considered equivalent to *Pneumocystis* DHPS mutations reduced the susceptibility of the mutant *S. cerevisiae* DHPS to sulfa drugs [6,7]. Given the paucity of effective PCP treatment regimens, the development of TMP-SMX or dapsone (used in combination with TMP-SMX for treatment) resistance would have significant clinical consequences. Since *Pneumocystis* cannot be cultured, studies of putative sulfa drug resistance have focused on correlating DHPS mutations with clinical variables. Most but not all of these studies have found a significant association between sulfa prophylaxis with either TMP-SMX and/or dapsone and the presence of DHPS mutations [8]. However, whether the effect of TMP-SMX and dapsone on DHPS mutations is similar or different is unknown.

In contrast, studies correlating the presence of DHPS mutations and mortality have had conflicting findings. Non-uniform mortality endpoints may be partially responsible for these conflicting results. Three of nine studies examining the impact of DHPS mutations on death have found a significantly increased risk for death at four weeks [9,10] or at three months [11] while one study observed an unexpected trend towards decreased mortality at six weeks [12]. Efforts to pool or compare previously collected data have been hampered by the different mortality endpoints used in these prior studies, perhaps explaining why the clinical significance of *Pneumocystis* mutations remains unclear.

While different definitions have been used for sulfa prophylaxis and clinical outcomes, the classification schema of mutant DHPS genotypes has been consistently applied in all studies. To date, all studies have compared mutant DHPS to wild-type DHPS and have defined mutant as ‘any mutant’ inclusive of both pure mutant and mixed infections, with pure mutant defined as a specimen with only one mutant DHPS genotype and mixed infection as a specimen harboring at least two DHPS genotypes, including at least one mutant genotype. However, it is unclear whether the decision to group pure mutant and mixed infections together is consistent with its ‘phenotype.’

To examine whether the variable definitions for prophylaxis and mortality endpoints and whether grouping pure mutant and mixed infections together could explain the discrepant results of prior studies, we used a 10-year prospective study of HIV-infected patients admitted to San Francisco General Hospital (SFGH) with PCP. We determined how the risk of DHPS mutations and death changed by applying different definitions of prophylaxis, mortality endpoints, and DHPS mutant. With the substantial heterogeneity found in published studies and the resultant inability to pool these data, this large single-center study represents an important opportunity to explore these questions.

Methods

Patients

We enrolled consecutive HIV-infected adults hospitalized with microscopically-confirmed PCP between May 1997 and February 2008. The study protocol was approved by the Institutional Review Boards at the University of California, San Francisco, the Centers for Disease Control and Prevention (CDC), and the University of North Carolina (UNC). The cohort included 372 episodes from 342 patients, of which 215 episodes were previously reported [13].

Clinical Data and Specimens

Data were collected via patient interview and chart abstraction using standardized data collection forms. PCP was microscopically confirmed using a modified Giemsa stain (Diff-Quik) on respiratory samples obtained from sputum induction or bronchoalveolar lavage (BAL) performed during routine clinical care. Respiratory specimens (one mL of sputum or BAL) were sent to the CDC (May 1997 to July 2002) or to UNC (August 2002 to February 2008) for genotyping via direct sequencing of amplicons generated via a nested PCR protocol targeting the relevant loci in DHPS, as described previously [14]. For patients with multiple episodes of PCP during the study period, only clinical and laboratory data from the last episode of PCP were included in the analysis.

Definition and Classification of DHPS Mutant

As in prior studies, the wild-type DHPS genotype (GenBank Accession Numbers: U66279) was defined as threonine at position 55 (Thr55) and proline at position 57 (Pro57); a mutant genotype was defined as any DNA sequence that differed from the wild-type such that alanine at position 55 (Ala55) and/or serine at position 57 (Ser57) were substituted [2,15]. We further sub-classified DHPS mutations as ‘pure mutants’ if only one mutant genotype was identified (Ala55, Ser57, or Ala55+Ser57) or ‘mixed infections’ if at least two genotypes were identified, including at least one mutant genotype; chromatographs were manually inspected at the loci of interest, and secondary peaks >20% of the major peak were scored as mixed [14]. Unlike prior studies, we analyzed pure mutant and mixed infections separately, but similar to prior studies, we also combined pure and mixed infections (‘any mutant’).

Definitions of Prophylaxis and Clinical Outcomes

Prior studies combined TMP-SMX or dapsone prophylaxis into 'sulfa/sulfone prophylaxis' [13]. In this study, we defined sulfa prophylaxis as TMP-SMX or dapsone prophylaxis use within 12 weeks preceding PCP diagnosis, irrespective of duration, and examined each chemoprophylaxis regimen separately and combined. We examined death (all-cause and PCP death) at three different endpoints used in prior studies: within four, six and 12 weeks after PCP diagnosis. Investigators blinded to DHPS genotype collected patient vital status and cause of death using SFGH databases and the National Death Registry. Death was classified as PCP death when the physician recorded PCP as the primary cause of death.

Statistical Analysis

First, DHPS mutant groups (any mutant, pure mutant or mixed infections) were analyzed as outcomes with prophylaxis as the predictor. Logistic analysis was used to test mutant vs. wild-type. Generalized logits regression with a multinomial distribution was used to test pure mutant vs. mixed mutant vs. wild-type. PCP prophylaxis was treated first as a binary predictor (any sulfa/sulfone vs. none), then as a 3-category predictor (TMP-SMX vs. dapsone vs. none); adjustment was made for prior PCP as a potential confounding factor. Second, mortality endpoints were analyzed as outcomes with mutant group and prior PCP as predictors. We used Kaplan-Meier survival analysis to estimate the cumulative incidence of survival at four, six, and 12 weeks dating from the first respiratory specimen of the last episode of PCP. We compared the equality of survivor functions with respect to DHPS subtype using the log-rank test. Cox regression was used to calculate hazard of death at different follow-up time-intervals dated from the last episode of PCP, adjusting for initial PCP treatment (sulfa/sulfone vs. non-sulfa/-sulfone treatment) and CD4 cell-count. Statistical significance was defined as p-value<0.05. Statistics were performed using SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

During the 10-year study period, 342 HIV-infected patients with 372 episodes of PCP were enrolled. DHPS genotyping was successful in 325 samples. There were no significant differences in clinical characteristics or outcomes between patients whose specimens could and could not be sequenced. Twenty patients had two or more episodes of PCP during the study period; as we were interested in examining mortality, we only included the last PCP episode for these patients. Therefore, 301 patients with a single episode of PCP during the study period were included in the analysis (Figure 1).

Patient Characteristics

Overall, the study population was mostly male (90%) with a mean age of 41 years (±8 years; Table 1). Patients in this cohort had advanced HIV disease with a median CD4 cell-count of 29 cells/μL (interquartile range [IQR] 13–64 cells/μL) and a mean log viral load of 11.8 copies/mL (±1.6 log copies/mL). Twenty-four percent of patients (71/301) were newly diagnosed with HIV infection during their hospitalization for PCP. A new diagnosis of HIV was significantly associated with DHPS genotype (Fisher p=0.003), with a significantly greater proportion of patients newly diagnosed with HIV having wild-type DHPS than pure

mutant DHPS (36% vs. 17%, generalized logits p=0.001). There were similar proportions of new HIV diagnosis in patients infected with wild-type DHPS compared to the mixed DHPS infection (36% vs. 29%, generalized logits p=0.35). When we compared the proportion of new HIV diagnosis by pure and mixed mutant subtype, a significantly greater proportion of patients infected with the mixed subtype were newly diagnosed with HIV compared to patients infected with the pure mutant subtype (29% vs. 17%, generalized logits p=0.04). Of note, none of the patients newly diagnosed with HIV reported having used sulfa prophylaxis with either TMP-SMX and/or dapsone prior to PCP diagnosis. There were no other significant differences in patient characteristics or PCP severity with respect to DHPS subtype.

DHPS Genotypes

Despite the infrequent utilization of sulfa prophylaxis in our cohort, only 69 patients (23%) possessed the DHPS wild-type as the sole genotype (Table 2). Compared to wild-type and mixed DHPS infections, pure mutant infections were the most common DHPS subtype (169/301, 56%), with the double mutant genotype (substitutions in positions 55 and 57) being the most common DHPS genotype (159/301, 53%). Sixty-three specimens (21%) were classified as mixed infections, in which at least two DHPS genotypes were present.

Prophylaxis as a Predictor of DHPS Mutation

Only 28% (85/301) of patients reported having used sulfa prophylaxis: 48 patients reported having used TMP-SMX, 36 patients reported having used dapsone and one patient reported having used both TMP-SMX and dapsone prophylaxis during the 12 weeks prior to PCP diagnosis. Of note, the proportion of patients reporting sulfa-prophylaxis use showed no significant annual variation during the 10-year study period (Poisson test for trend over time p=0.78). When we applied the prior definition of sulfa prophylaxis (sulfa/sulfone prophylaxis) to the prior definition of DHPS mutant (any mutant) and adjusted for prior PCP history, prophylaxis was significantly associated with any mutant (adjusted odds ratio [AOR]=2.87, p=0.01; Table 3). When we examined TMP-SMX and dapsone prophylaxis separately, dapsone (AOR=5.83, p=0.02) but not TMP-SMX (AOR=1.98, p=0.13) was significantly associated with any mutant. However, when we compared dapsone to TMP-SMX prophylaxis with respect to the likelihood of infection with any mutant, we found that dapsone was not more likely than TMP-SMX to be associated with any mutant infection (OR=2.94, p=0.20).

When DHPS mutant subtypes (pure and mixed) were examined separately, prophylaxis, irrespective of the definition used, was significantly associated with the pure mutant subtype (all p<0.04; Table 3). In contrast, prophylaxis, irrespective of the definition used, was not associated with mixed infections. Similar to patients reporting prophylaxis use, patients with prior PCP were more likely to be infected with the pure mutant subtype than patients without this history. However, prior PCP was not associated with either mixed infections or infection with any DHPS mutant.

DHPS Mutation as a Predictor of Death

Four-, six-, and 12-week vital status was known 296, 295 and 293 patients, respectively. There were 24 deaths (20 due to PCP) within four weeks following PCP diagnosis, 32 deaths (23 due to PCP) within six weeks following PCP diagnosis, and 42 deaths (25 due to PCP) within 12 weeks following PCP diagnosis. Death (all-cause and PCP-attributed mortality) was examined relative to DHPS mutant status adjusting for initial PCP treatment (sulfa/sulfone vs. non-sulfa/-sulfone) and CD4 cell-count. Comparing wild-type to any DHPS mutant, the cumulative incidence of four-week (4% vs. 9%, difference −5%, 95% CI: −31 to +21%, p=0.78), six-week (4% vs. 12%, difference −8%, 95% CI: −34 to +18%, p=0.67), and 12-week all-cause mortality (9% vs. 16%, difference −7%, 95% CI: −32 to +19%, p=0.66) was similar to infection with any mutant (Figure 2A). Similarly, there was no difference in the cumulative incidence of four-week (3% vs. 8%, difference −5%; 95% CI: −31 to +22%, p=0.80), six-week (3% vs. 9%, difference −6%; 95% CI: −32 to +21%, p=0.77), and 12-week PCP-attributed mortality (4% vs. 9%, difference −5%; 95% CI: −31 to +21%, p=0.77) when we compared wild-type infections to infection with any mutant (Figure 2C).

When we examined mortality with respect to DHPS subtype (wild-type vs. pure mutant vs. mixed infection), there was also no difference in cumulative incidence of four-, six- and 12-week all-cause mortality (all p>0.61, Figure 2B) or PCP-attributed mortality (all p>0.75, Figure 2D). Additionally, infection with a DHPS mutant genotype (any, pure or mixed) was not associated with a changing hazard for death over time (all p>0.06, Table 4).

Discussion

Applying different definitions of sulfa prophylaxis, mortality endpoints and DHPS mutant to our 10-year cohort, there were three main findings. First, prophylaxis, irrespective of the definition used, was significantly associated with pure DHPS mutant infection. Second, prophylaxis, irrespective of the definition used, was not associated with mixed DHPS infection. Finally, infection with *P. jirovecii* containing a DHPS mutant genotype was not associated with increased mortality, irrespective of cause of death or DHPS mutant subtype (pure or mixed) nor was infection with a DHPS mutant (any, pure or mixed) associated with a changing hazard for death over time.

Consistent with most prior studies, we found an increased risk of DHPS mutants among patients reporting sulfa prophylaxis [8,16]. This association appears driven by the selective pressure of prophylaxis favoring a pure mutant infection rather than the specific choice of prophylaxis. Only four studies have found no association with sulfa prophylaxis and DHPS mutant. Interestingly, all four studies may have defined sulfa prophylaxis differently from those definitions used in this study: two studies did not define prophylaxis duration [17,18] while two studies defined prophylaxis as sulfa use for a minimum of one week in the six months preceding PCP diagnosis [9,19]. Furthermore, one study observed a higher prevalence of mixed infections relative to pure mutant infections [9]. Combined with our study findings, this suggests that differing definitions of prophylaxis as well as differing proportions of pure mutant and mixed infections in earlier cohorts may have contributed to the conflicting associations observed in prior studies.

Whether PCP is acquired from an environmental source or from person-to-person transmission, *P. jirovecii* is a ubiquitous organism and repeated exposure over a lifetime is likely. Therefore, with repeated exposures, an HIV-infected, sulfa-naïve patient could harbor multiple *Pneumocystis* DHPS genotypes. Consistent with this theory, our study found a higher proportion of mixed infections among those patients without prior sulfa-drug exposure. This observation is also consistent with the findings of a large multi-center study by Beard *et al*, who reported primary PCP, as well as city of residence, are independent risk factors for mixed mutant infections [20]. Conversely, subsequent sulfa exposure would exert a selective pressure favoring one genotype over another, resulting in infection with a single mutant genotype. Indeed, two studies have found an increasing prevalence of DHPS mutant infection since sulfa prophylaxis was first recommended for PCP prevention for people living with HIV in 1989 [2,11].

Whether *Pneumocystis* DHPS mutant infection is associated with important clinical outcomes has been the subject of an even greater debate as prior attempts to answer this question have yielded conflicting results. Despite our investigation of two mortality outcomes (all-cause and PCP death), three different mortality endpoints (four, six and 12 weeks post-PCP diagnosis), and multiple DHPS classification schema, there were no associations between DHPS mutant infection and mortality. Two studies have shown that mutations at the DHPS loci confer only modest sulfa drug resistance [6,7]; the fact that most patients infected with *Pneumocystis* DHPS mutants are successfully treated with high-dose sulfa therapy is consistent with the lack of mortality difference observed in our study. There are however, a few potential reasons why *Pneumocystis* DHPS mutant infection was not associated with a significant increase in mortality in our study. First, our study had a higher prevalence of mixed DHPS infections (21%) compared to prior studies that have observed an association between DHPS mutant infection and death (prevalence range: 0–3%) [9–11]. The higher prevalence of mixed infections observed in our study may have potentially impacted a mortality difference from being observed. Second, the proportion of patients newly diagnosed with HIV was highest among the wild-type subtype. Although several studies have reported DHPS mutant infection to be associated with increased PCP severity [2,9,11,13], patients unaware of their HIV diagnosis may delay seeking medical care, presenting at a later and more advanced stage of PCP. Therefore, the higher proportion of newly diagnosed HIV-infected patients among those with the wild-type genotype may have inflated mortality in this group and masked a significant mortality difference between DHPS subtypes. Third, despite this study representing the single-largest cohort of HIV-infected patients with PCP to examine the impact of DHPS mutant infection on death, cumulative mortality was low, potentially limiting our ability to detect a significant difference in mortality. Finally, mortality in HIV-infected persons with PCP is complex and is affected not only by the severity of the disease but also by factors such as the severity of HIV/AIDS, the presence of co-morbidities, and development of complications. Standardizing methodological variables (predictors and outcomes) in addition to evaluating individual DHPS subtypes, perhaps even individual genotypes, would allow pooling of existing data-sets from different study sites across different time periods, to more definitively address this issue.

Our study had limitations. First, there is no universally accepted nomenclature for DHPS mutants; categorization into pure mutant and mixed subtypes is a new classification without a standard for comparison. Our study findings suggest, however, that pure and mixed subtypes behave differently and should thus be considered separately in future studies. Additionally, the selective effect of sulfa prophylaxis on infection with a pure mutant genotype offers an explanation for the conflicting findings of previous studies. Second, our study did not include all previously-used definitions of prophylaxis and mortality endpoints used in earlier studies; we chose to limit the definitions of predictor and outcome to those most commonly used, including those used in studies with conflicting findings. Third, our study lacked post-mortem data which would have provided more accurate assignment of cause of death in the study population and on whether DHPS mutant infection was associated with PCP-attributed mortality. Lastly, as a single-center cohort study, our study findings may not be applicable to other populations. However, because rates of losses to follow-up were low (12-week vital status known for 97.3% of patients) and because rates of losses to follow-up were similar by DHPS subtype (any, pure and mixed), any biases secondary to informative censoring are likely to be small.

In conclusion, the major findings of our study are: 1) prophylaxis, irrespective of the definition used, was strongly associated with pure mutant infection; 2) prophylaxis was not predictive of mixed infections; and 3) infection with *Pneumocystis* DHPS mutant was not associated with increased mortality, irrespective of DHPS mutant subtype, cause of death or mortality endpoint used. Future studies should examine individual prophylaxis regimens with respect to DHPS mutant group and standardize clinical outcomes measured thus allowing subsequent results to be pooled as larger studies are likely needed to address this issue comprehensively. Furthermore, DHPS subtypes, perhaps even individual genotypes, should be examined separately with respect to both predictors and outcomes.

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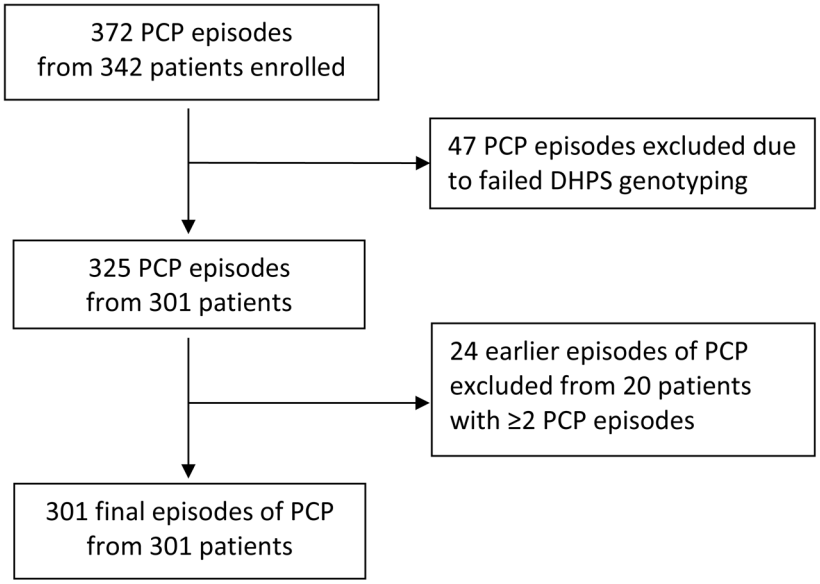

Figure 1. Study enrollment

Overall, 342 HIV-infected patients with 372 episodes of PCP were enrolled during the study period. DHPS genotyping was unsuccessful for 47 episodes of PCP and were thus excluded, leaving 325 cases of PCP from 301 patients. Twenty patients experienced two or more episodes of PCP during the study period. Because only the last episode of PCP was included in this analysis, we excluded 24 prior cases of PCP. Therefore, 301 patients with a single episode of PCP during the study period were examined in this study.

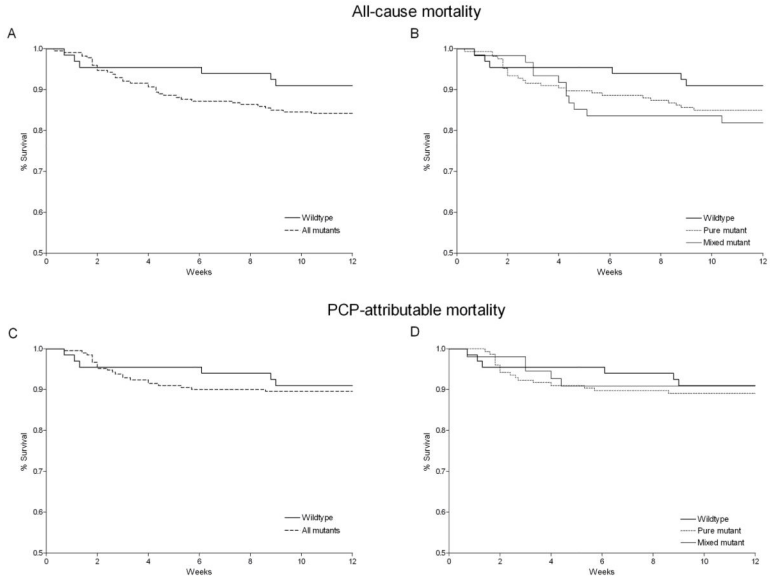

Figure 2.

Figure 2A. Survival of HIV-infected patients with *Pneumocystis* pneumonia: wild-type vs. any DHPS mutant.

Kaplan-Meier survival curves are shown for HIV-infected patients diagnosed with *Pneumocystis* pneumonia with and without DHPS mutations. Compared to wild-type DHPS infection, the cumulative incidence of all-cause mortality was similar to infection with any mutant (includes pure and mixed subtypes): at four weeks (4% vs. 9%, p=0.78), six weeks (4% vs. 12%, p=0.67), and 12 weeks mortality (9% vs. 16%, p=0.66).

Figure 2B. Survival of HIV-infected patients with *Pneumocystis* pneumonia: wild-type vs. pure DHPS mutant subtype vs. mixed DHPS mutant subtype.

Kaplan-Meier survival curves are shown for HIV-infected patients diagnosed with *Pneumocystis* pneumonia with three DHPS subtypes: wild-type, pure mutant and mixed mutant. There was no difference in all-cause mortality at four (wild-type vs. pure: 4% vs. 9%, p=0.77; wild-type vs. mixed: 4% vs. 8%, p=0.84), six (wild-type vs. pure: 4% vs. 11%, p=0.72 ; wild-type vs. mixed: 4% vs. 16%, p=0.61) and 12 weeks (wild-type vs. pure: 9% vs. 15%, p=0.70; wild-type vs. mixed: 9% vs. 17%, p=0.62) following PCP diagnosis when DHPS mutant subtypes (pure and mixed) were examined separately.

Figure 2C. Survival of HIV-infected patients with *Pneumocystis* pneumonia: wild-type vs. any DHPS mutant.

Kaplan-Meier survival curves are shown for HIV-infected patients diagnosed with *Pneumocystis* pneumonia with and without DHPS mutations. Compared to wild-type infection, the cumulative incidence of PCP-attributable mortality was similar to infection with any mutant (includes pure and mixed subtypes): at four weeks (3% vs. 8%, p=0.80), six weeks (3% vs. 9%, p=0.77), and 12 weeks (4% vs. 9%, p=0.77).

Figure 2D. Survival of HIV-infected patients with *Pneumocystis* pneumonia: wild-type vs. pure DHPS mutant subtype vs. mixed DHPS mutant subtype.

Kaplan-Meier survival curves are shown for HIV-infected patients diagnosed with *Pneumocystis* pneumonia with three DHPS subtypes: wild-type, pure mutant and mixed mutant. There was no difference in PCP-attributable mortality at four (wild-type vs. pure: 3% vs. 8%, p=0.79; wild-type vs. mixed: 3% vs. 6%, p=0.86), six (wild-type vs. pure: 3% vs. 9%, p=0.76; wild-type vs. mixed: 3% vs. 8%, p=0.81), and 12 weeks (wild-type vs. pure: 4% vs. 10%, p=0.75; wild-type vs. mixed: 4% vs. 8%, p=0.84) following PCP diagnosis when DHPS mutant subtypes (pure and mixed) were examined separately.

Table 1

Demographics and clinical characteristics

Characteristic, N (%)	Overall	DHPS subtype		
		Wild-type	Pure mutant	Mixed mutant
N (% of total)	301 (100%)	69 (23%)	169 (56%)	63 (21%)
Demographic data				
Mean age (years [SD])	41 (8)	41 (8)	40 (7)	42 (9)
Male	270 (90%)	63 (91%)	147 (87%)	60 (95%)
Race/ethnicity				
White	147 (49%)	37 (54%)	74 (44%)	36 (57%)
African-American	89 (30%)	16 (23%)	56 (33%)	17 (27%)
Hispanic/Latino	45 (15%)	9 (13%)	27 (16%)	9 (14%)
Other	20 (7%)	7 (10%)	12 (7%)	1 (2%)
HIV characteristics				
*New HIV diagnosis	71 (24%)	25 (36%)	28 (17%)	18 (29%)
Median CD4 count (cells/μL [IQR])	29 (13–64)	35 (13–76)	25 (10–60)	40 (18–64)
Mean log viral load (SD)	11.8 (1.6)	11.6 (1.4)	11.8 (1.5)	11.8 (1.7)
PCP characteristics				
Mean serum albumin (g/L [SD])	3.0 (0.6)	3.1 (0.6)	3.0 (0.6)	3.0 (0.5)
Median serum LDH (U/L [IQR])	361 (270–522)	367 (267–516)	355 (265–522)	368 (306–541)
Intensive care unit	38 (13%)	6 (9%)	22 (13%)	10 (16%)
Mechanical ventilation	33 (11%)	4 (6%)	21 (12%)	8 (13%)

ABBREVIATIONS: DHPS, dihydropteroate synthase; SD, standard deviation; IQR, interquartile range; PCP, *Pneumocystis* pneumonia; LDH, lactase dehydrogenase.

NOTE: All comparisons p>0.05 except for *New HIV diagnosis, Fisher p=0.003.

Table 2

Genotype analysis of *Pneumocystis jirovecii* isolates at the DHPS locus of 301 HIV-infected patients with *Pneumocystis* pneumonia

DHPS genotype	Amino acid at position 55	Amino acid at position 57	N (%)
Wild-type	Threonine	Proline	69 (23%)
Any mutant			232 (77%)
Pure mutant position 55	Alanine	Proline	6 (2%)
Pure mutant position 57	Threonine	Serine	4 (1%)
Pure mutant positions 55 & 57	Alanine	Serine	159 (53%)
Mixed mutant	Threonine/Alanine	Proline/Serine	63 (21%)

ABBREVIATIONS: DHPS, dihydropteroate synthase.

Table 3

Prophylaxis and history of prior *Pneumocystis* pneumonia as predictors of DHPS mutant infection

	Any Mutant		Pure Mutant		Mixed Mutant	
	AOR (95% CI)	**p-value**	**AOR (95% CI)**	**p-value**	**AOR (95% CI)**	**p-value**
No prophylaxis (N=213)	1.00		1.00		1.00	
* Any prophylaxis (N=84)	2.87 (1.33–6.19)	0.01	4.00 (1.83–8.76)	<0.001	0.78 (0.26–2.36)	0.65
† TMP-SMX prophylaxis (N=48)	1.98 (0.82–4.76)	0.13	2.58 (1.05–6.32)	0.04	0.85 (0.25–2.88)	0.79
† Dapsone prophylaxis (N=35)	5.83 (1.35–25.2)	0.02	8.84 (2.02–38.8)	0.004	0.56 (0.05–6.48)	0.64
‡ Dapsone vs. TMP-SMX	2.94 (0.57–15.2)	0.20	3.43 (0.65–18.1)	0.15	0.67 (0.05–9.65)	0.76
No history of PCP (N=229)	1.00		1.00		1.00	
Prior history of PCP (N=67)	1.81 (0.82–3.99)	0.14	2.56 (1.14–5.73)	0.02	0.47 (0.14–1.65)	0.42

ABBREVIATIONS: DHPS, dihydropteroate synthase; AOR, Adjusted Odds Ratio; CI, Confidence Interval; TMP-SMX, trimethroprim-sulfamethoxazole; PCP, *Pneumocystis* pneumonia.

NOTE:

*Adjustments made for prior PCP.

†Excluding 4 patients for whom a prior PCP history was unknown, N=297.

‡Excluding 1 patient who used both TMP-SMX and dapsone within 12 weeks prior to admission, N=296.

Table 4

DHPS subtype as a predictor of death

	Dead at 4 weeks			Dead at 6 weeks			Dead at 12 weeks		
	N (%)	**AHR (95% CI)**	**p-value**	**N (%)**	**AHR (95% CI)**	**p-value**	**N (%)**	**AHR (95% CI)**	**p-value**
All-cause death	24 (8)			32 (11)			42 (14)		
DHPS									
Wild-type	3 (4)	1.00		3 (4)	1.00		6 (9)	1.00	
Any mutant	21 (9)	1.84 (0.54–6.19)	0.33	29 (13)	2.65 (0.80–8.72)	0.11	36 (16)	1.62 (0.68–3.86)	0.28
DHPS									
Wild-type	3 (4)	1.00		3 (4)	1.00		6 (9)	1.00	
Pure mutant	16 (9)	1.93 (0.56–6.69)	0.30	19 (11)	2.33 (0.68–7.90)	0.18	25 (15)	1.47 (0.60–3.60)	0.40
Mixed	5 (8)	1.55 (0.35–6.95)	0.57	10 (16)	3.60 (0.97–13.4)	0.06	11 (17)	2.12 (0.77–5.88)	0.15
PCP death	20 (7)			23 (8)			25 (8)		
DHPS									
Wild-type	2 (3)	1.00		2 (3)	1.00		3 (4)	1.00	
Any mutant	18 (8)	2.36 (0.54–10.2)	0.25	21 (9)	2.81 (0.66–12.1)	0.16	22 (9)	1.97 (0.59–6.61)	0.27
DHPS									
Wild-type	2 (3)	1.00		2 (3)	1.00		3 (4)	1.00	
Pure mutant	14 (8)	2.59 (0.58–11.5)	0.21	16 (9)	2.98 (0.68–13.1)	0.15	17 (10)	2.10 (0.61–7.21)	0.24
Mixed	4 (6)	1.71 (0.28–10.2)	0.56	5 (8)	2.33 (0.43–12.8)	0.33	5 (8)	1.58 (0.35–7.10)	0.55

ABBREVIATIONS: DHPS, dihydropteroate synthase; AHR, adjusted hazard ratio; CI, confidence interval.

Adjustments made for *Pneumocystis* pneumonia treatment and CD4 cell-count.