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Infectious Disease Practice

N-acetylcysteine modulates markers of oxidation, inflammation and infection in tuberculosis

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SUMMARY

Background: Half the global tuberculosis health burden is due to post-tuberculosis lung disease. Host-directed therapies have been proposed to reduce this burden. N-acetylcysteine (NAC) provides the conditionally essential amino acid cysteine required for synthesis of glutathione, an antioxidant thiol. We recently reported clinical outcomes of a trial of adjunctive NAC in patients with pulmonary tuberculosis, finding that NAC improved the secondary endpoint of recovery of lung function. Here we report the effects of NAC on biomarkers of oxidation, inflammation, and infection in that trial.

Methods: 140 adults with moderate or far-advanced pulmonary tuberculosis were randomly assigned to standard tuberculosis treatment with or without NAC 1200 mg twice daily for months 1–4. Sputum and blood samples were obtained at specified intervals to measure total glutathione, MTB-induced cytokines, hemoglobin, whole blood mycobactericidal activity (WBA), and sputum MTB burden.

Results: NAC treatment rapidly increased total glutathione ($P < .0001$), but levels did not reach those of healthy volunteers ($P < .001$). NAC reduced MTB-induced TNF-α ($P = .011$) without affecting IL-10, and accelerated the recovery of hemoglobin in participants with low values on entry. NAC did not affect killing in *ex vivo* whole blood culture but did slow the clearance of MTB from sputum ($P=0.003$).

Conclusion: Adjunctive NAC showed antioxidant and anti-inflammatory effects consistent with the amelioration of immunopathology seen in preclinical models. Two biomarkers of antimicrobial activity showed discordant results; neither demonstrated the enhanced antimicrobial effects seen preclinically. The reduction of oxidative stress and inflammation by NAC may explain its effects on the recovery of lung function post-TB.

Introduction

Tuberculosis (TB) has remained a major global health concern despite concerted efforts toward its elimination.[1] Recent studies have confirmed that a substantial burden of cardiorespiratory illness persists despite ostensibly successful tuberculosis treatment.[2–4]

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Adjunctive host-directed therapeutics (HDTs) have been proposed to address this unmet need.[5] One such candidate, N-acetylcysteine (NAC), has been in clinical use since the 1970s to prevent fatal hepatic necrosis following acetaminophen poisoning.[6] Mucolytic, antioxidant, anti-inflammatory, and antimicrobial properties have been described, including beneficial effects on mycobacterial colony counts and immunopathology in preclinical tuberculosis models.[7,8] Some of the therapeutic effects of NAC have been attributed to replenishing cellular glutathione during severe or sustained oxidative stress. NAC may also enhance the antimicrobial activity of certain antituberculosis drugs.[9–11]

We recently completed a randomized controlled trial examining the effects of adjunctive NAC on clinical and microbiologic TB treatment outcomes.[12] Although NAC improved the recovery of lung function (a secondary outcome of the trial), we found no effect on the microbiologic outcomes of the hazard ratio (HR) for stable culture conversion (its primary outcome) or the odds ratio (OR) for recurrence. Indeed, point estimates for both parameters were contrary to anticipated results, though neither reached statistical significance (HR=0.84, 95%CI=0.59–1.20, P=0.33; OR=2.58, 95%CI=0.51 to 14.49, P=0.27). The finding that both endpoints showed similar deleterious trends raised the potential concern that NAC might impair rather than enhance the eradication of MTB infection, possibly by interfering with host defense mechanisms of innate immune effector cells mediated by reactive oxygen species (ROS). The studies in this current report were undertaken to better understand the potential mechanisms of these findings. We sought specifically to examine the anti-inflammatory and microbiologic effects of NAC in greater detail.

Methods

Study design and participants

This trial was a phase 2 open-label prospective parallel-arm randomized controlled trial conducted at the Mbeya Medical Research Centre of the National Institute for Medical Research in Mbeya, Tanzania, as a nested sub-study of the TB SEQUEL cohort study.[13] Participants were persons aged 18–65 years providing written informed consent, with initial episodes of rifampin-susceptible pulmonary tuberculosis diagnosed by molecular testing, chest radiography showing moderately or far advanced pulmonary tuberculosis,[14] and body mass of 30–90 kg. Ethical approval was granted by the Mbeya Medical Research and Ethics Committee, and the Medical Research Coordinating Committee of Tanzania. Regulatory approval was granted by the Tanzania Medicines and Medical Devices Authority. Data and safety were monitored by an external committee of experts. The trial was registered at Clinicaltrials.gov as NCT03702738.

Randomization and treatment

Participants were randomly assigned to NAC 1200 mg BID from days 1–112, or control, at a ratio of 1:1, with stratification according to radiographic extent of disease.[14] All participants received standard 4-drug TB treatment (2HRZE/4HR) using fixed-dose combination tablets. 600 mg NAC capsules were donated to the study by NOW Foods (www.nowfoods.com). Enrolled participants were examined at regular intervals through day 168 for medical history review, physical examination, spirometry, and collection of blood and sputum samples. After completion of sub-study participation, patients returned to the TB-SEQUEL main cohort for extended follow-up. A separate protocol described the recruitment of an additional cohort of volunteers to establish a local reference range of expected glutathione concentrations in whole blood samples of healthy individuals. This protocol was also approved by the Mbeya Medical Research and Ethics Committee. Volunteers were recruited between June and August 2021 from among medical center personnel. None had a history of tuberculosis or malaria during the 6-month period prior to testing.

Mycobacterial Growth Indicator Tube Time to Positivity (MGIT TTP)

Time to detection of growth of *M tuberculosis* in automated liquid culture (MGIT TTP) is inversely related to the log of the inoculum; TTP increases as the mycobacterial burden in sputum declines during treatment. TTP values were recorded for each sputum sample from screening through day 56. The mean of screening and day 0–1 cultures was taken as the baseline value. The first instance of a culture negative for growth of MTB for any participant was scored as 42 days; subsequent negative cultures were censored, as were contaminated cultures. Trends over time were examined by linear mixed effects modeling, with treatment arm and day as fixed effects, and participant as a random effect.

Whole blood bactericidal activity (WBA)

This technique measures the mycobactericidal activity of blood sampled before and at intervals after immunologic or antibacterial treatment is administered, by inoculating the blood samples with a reference *M. tuberculosis* isolate. WBA was measured on study day 1 (prior to the start of antituberculosis treatment), day 14 (during the intensive phase of treatment), and day 84 (during the continuation phase). On day 1, the first dose of NAC was administered at the study clinic 2 h prior to initiation of anti-tuberculosis treatment, to distinguish direct NAC effects from those due to GSH. Blood was sampled prior to, and at 1 and 2 hours post-NAC dose. This time period was selected based on the short time to peak drug concentration (Tmax, 1 hr) and the short elimination half-life (T½, 2.4hrs) of NAC after oral dosing.[15] Blood samples were drawn from untreated control participants over a similar time period. On days 14 and 84, NAC and antimicrobial drugs were administered simultaneously. Blood samples were obtained at 0, 2, 4, and 6 hrs post dose. Heparinized blood collected at specified intervals was stored at room temperature with slow constant rotation until all samples from that participant were collected, at which time they were transported to the laboratory for testing. WBA against *M. tuberculosis* H37Rv was determined as previously described.[16] Briefly, *M tuberculosis* H37Rv was grown in MGIT and frozen in aliquots at −80ºC. A titration experiment determined the relationship between inoculum size and MGIT TTP, and identified the volume positive in 5.5 days. Whole blood cultures consisted of 300 µl heparinized blood, an equal volume of RPMI 1640 tissue culture medium with l-glutamine and 4-(2-hydroxyethyl)piperazine-1-ethane-sulfonic acid (HEPES, Gibco), and mycobacteria from the specified volume of stock. After 72 h incubation at 37 ºC with slow rotation, whole blood cultures were removed from the incubator and cells were sedimented. The liquid phase was removed, sterile filtered, and frozen at −80ºC for later cytokine analysis. The sedimented blood cells were then disrupted by hypotonic lysis. Bacilli were recovered and inoculated into MGIT and incubated until flagged as positive. Log change in viability was calculated as log(*final*) – log(*initial*), where *final* and *initial* are the volumes corresponding to the TTP of the completed cultures and its inoculum control, respectively, based on the titration curve. Results were expressed as log change per day of whole blood culture (Δlog/d), with positive values indicating growth. Cumulative WBA was calculated as the AUC_{0-24} using the trapezoidal method, assuming steady-state conditions (*ie*, equality at 0 and 24hrs). Results were expressed as Δlog/d·d, or simply as log change.

Cytokine analysis

TNF and IL-10 were measured in reserved whole blood culture supernatants by colorimetric ELISA (R&D Systems MN 55413 USA) as recommended by the manufacturer.

Glutathione

Total glutathione (the sum of reduced and oxidized forms) was measured in heparinized whole blood samples using a colorimetric assay (Arbor Assays, Ann Arbor USA) according to the manufacturer's instructions. In this assay, oxidized glutathione (GSSG) is reduced prior to detection, such that each molecule of oxidized glutathione contributes 2 molecules of GSH to those already existing in a sample as free (reduced) GSH. Total glutathione is reported here according to its GSH content.

Statistical analysis

The mean result of replicate samples was taken as the true value. Values in the experimental and control arms were compared by t test, or, if not normally distributed, by log-rank test. Linear mixed-effects models were used to analyze repeated measures. In some analyses, values were log transformed to improve linearity. The models fit a random intercept for each participant and examined the interaction between the treatment arm and time (arm*time) to assess differences between the study arms emerging over time.

Role of the funding source

Funding for this study was provided by the German Ministry of Education and Research (BMBF). Aside from approving the original study proposal, BMBF had no role in determining study design; data collection, analysis, and interpretation; in the writing of the report; or in the decision to submit this paper for publication. The Sponsor (The Aurum Institute) had no financial or other interest in the study intervention (NAC).

Results

Baseline characteristics

Screening, enrollment, and participation are shown in Supplemental Fig. s1. Baseline characteristics of the mITT study population and healthy volunteers are described in Supplemental table s1. Study participants were predominantly male and HIV uninfected, with a heavy sputum burden of infection. Nearly half had far advanced tuberculosis by chest radiography. There were no clinically significant differences between the two arms at baseline.

Glutathione

Baseline total glutathione concentrations are described in Supplemental table s1 and Fig. 1. Levels in patients were less than half those of healthy volunteers (P < .001). The evolution of GSH levels in trial participants over time is shown in Fig. 1. The recovery of glutathione in control participants was delayed and partial, remaining significantly less than those of healthy volunteers through the last measurement at month 6. Visual inspection of NAC responses revealed 3 distinct periods: a brisk initial response to day 14; a flattened subsequent response; and a decline to control levels after NAC treatment ended. We therefore used linear mixed effects models to separately examine days 1–14 and 14–112 (periods 1 and 2, respectively) during NAC treatment. Glutathione increased more rapidly in NAC than control participants during days 1–14, as shown by the interaction terms for day and study arm (Day*NAC arm) in

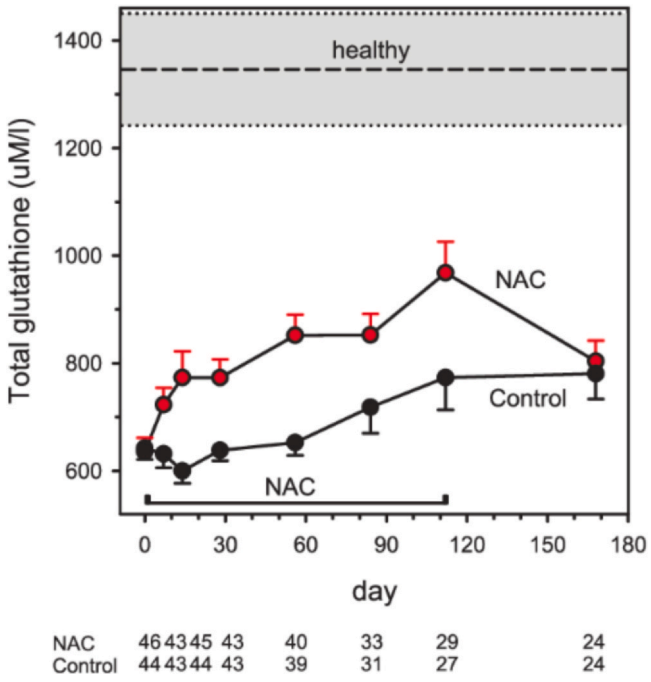

Fig. 1. Mean total whole blood glutathione according to study day and treatment arm. Numbers indicate participants at each time point. Error bars indicate SEM.

Table 1
Linear mixed effects modeling of total glutathione according to study day and treatment arm.

Source	Estimate	95% CI	P
Days 1–14			
Intercept	653.7	596.4 – 710.9	< 0.0001
Study day	−3.6	−7.9 – 0.6	0.1
Control arm	0.0		
NAC arm	−14.6	−95.6 – 66.4	0.7
Day*Control arm	0.0		
Day*NAC arm	13.8	7.7 – 19.9	< 0.0001
Days 14–112			
Intercept	577.9	513.9 – 642.1	< 0.0001
Study day	1.8	0.9 – 2.6	< 0.0001
Control arm	0.0		
NAC arm	160.2	69.5 – 250.9	0.001
Day*Control arm	0.0		
Day*NAC arm	0.1	−1.1 – 1.2	0.9

Glutathione levels increased in NAC recipients by 13.8 uM per day (Day*NAC arm) compared to controls from days 1–14. There was no further change from days 14–112.

Table 1 (P < .0001). This term, which reflects the difference in slope between the two arms during this period, indicates that NAC treatment had the desired effect on GSH synthesis. Levels remained elevated in NAC recipients from days 14–112 (P < .0001) but did not increase further relative to controls during this period (P=0.92). Glutathione in NAC recipients dropped to levels similar to controls after NAC treatment ceased.

Cytokine and hematologic effects

Levels of MTB-induced TNF and IL-10 in ex vivo whole blood culture were highest at the time of TB diagnosis and decreased progressively thereafter (Fig. 2). NAC treatment accelerated the decline in TNF (P=0.01) but had no effect on IL-10 (P=0.81, Table 2). Hemoglobin values < 13 g/dl were present at baseline in 84 of 140 participants. In this subset, analyzed post hoc, the recovery of hemoglobin levels proceeded more rapidly in NAC recipients (P < .05 at days 56 and 112, Fig. 3).

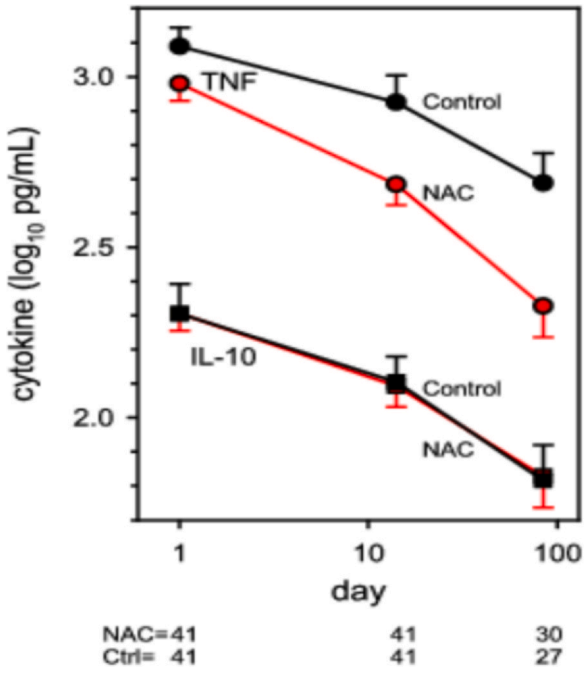

Fig. 2. Mean production of TNF and IL-10 in MTB-stimulated whole blood cultures, according to study arm and day. Error bars indicate SEM.

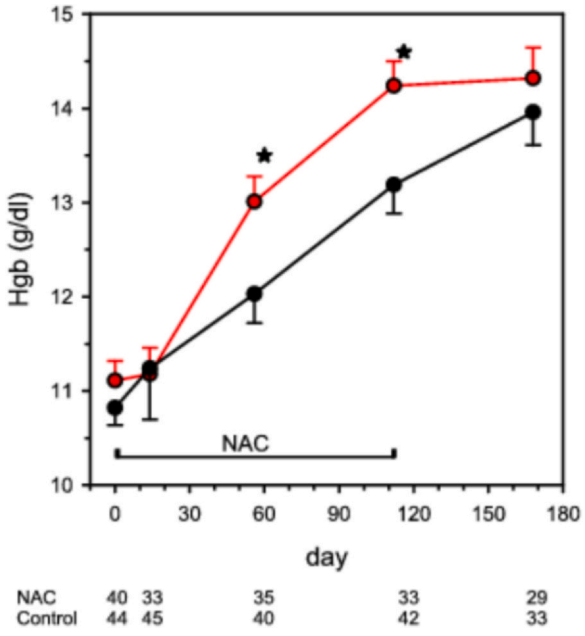

Fig. 3. Hemoglobin levels over time according to treatment arm, in participants with values < 13 g/dl on entry. Error bars indicate SEM. * indicates P < .05. Numbers indicate participants at each time point. Red dots indicated NAC recipients, and black dots indicate the control group.

Table 2
Linear mixed effects modeling of NAC effects on log MTB-induced TNF and IL-10, according to treatment arm and log study day.

Source	Value	95% CI	P
TNF (log pg/ml)			
Intercept	3.11	2.99 – 3.23	< 0.0001
log day	−0.21	−0.27 – −0.14	< 0.0001
Control arm	0.00		
NAC arm	−0.10	−0.27 – 0.06	0.22
Log Day*Control arm	0.00		
Log Day*NAC arm	−0.13	−0.22 – −0.04	0.01
IL-10 (log pg/ml)			
Intercept	2.32	2.17 – 2.48	< 0.0001
log day	−0.22	−0.30 – −0.14	< 0.0001
Control arm	0.00		
NAC arm	−0.01	−0.23 – 0.21	0.93
Log Day*Control arm	0.00		
Log Day*NAC arm	0.01	−0.10 – 0.13	0.81

Log TNF declined by −0.13 per log day in NAC recipients relative to controls. There was no effect on IL-10.

Whole blood bactericidal activity (WBA)

Mycobacteria added to whole blood cultures are rapidly ingested by neutrophils and monocytes.[17] Expression of cytokines and mediators by infected cells promote interactions that restrict *M. tuberculosis* growth.[18,19] Pre-treatment samples (0 hr) taken on day 1 showed a growth rate of *M. tuberculosis* $H_{37}Rv$ in whole blood culture of approximately 0.2 log/d, consistent with prior studies [20] (Fig. 4 left panel). This observed growth rate is about half that observed in broth culture, an effect attributable to innate and acquired host defenses.[17] On day 1, the first NAC dose was administered 2 hrs prior to the first doses of anti-tuberculosis drugs, so that its effect could be measured separately. This dose had no detectable effect on mycobacterial viability, whether measured as a change from baseline to 2 h (P=.38 by rank sum test) or by linear mixed effects modeling over the 2-hour period (P=0.22, Table 3).

Blood sampling for WBA was repeated on days 14 (during the intensive phase of treatment) and 84 (during the continuation phase, Fig. 4 center and right panels). On these occasions, NAC and anti-tuberculosis drugs were co-administered. These show the characteristic time-course profiles of isoniazid and rifampin, reaching maximum bactericidal activity 2–3 h post-dose. Treatment arms and periods were compared according to pre-dose (0 hr) and total (AUC_{0-24}) effects. Neither pre-dose values nor total effects differed between the NAC and control arms on days 14 or 84 (Table 4). However, pre-dose values showed superior killing on day 14 compared to 84 regardless of treatment arm (P < .001 for both). Values at this time point (prior to daily dosing) reflect the residual effects of the pyrazinamide dose of the previous day. Of the 4 standard anti-tuberculosis drugs, only pyrazinamide has a long elimination half-life (10 hrs).[21] Thus, we found no evidence that NAC either enhanced or interfered with drug effects or host defenses against intracellular *M. tuberculosis*.

MGIT TTP

The evolution of time to detection of MTB growth (MGIT TTP) in automated liquid cultures of sputum from baseline to day 56 is illustrated in Fig. 5. Responses over this time were sufficiently linear to permit a single analysis of the entire interval. Linear mixed effects modeling (Table 5) indicated that although TTP values in the NAC arm were 2.09 days greater than control at baseline (P=0.04), their rate of increase was slower than controls (Day*NAC arm, −0.11 TTP day per treatment day relative to control, P=0.003).

Discussion

This study examined the effects of adjunctive NAC on anti-inflammatory and antimicrobial biomarkers in adults with pulmonary tuberculosis. Its key findings are that NAC partially replenished glutathione; demonstrated immunologic and hematologic effects consistent with accelerated resolution of inflammation: However, Whole blood bactericidal assay and mycobacterial burden by sputum culture showed divergent effects on different markers of antimicrobial activity. These findings shed light on some of the clinical

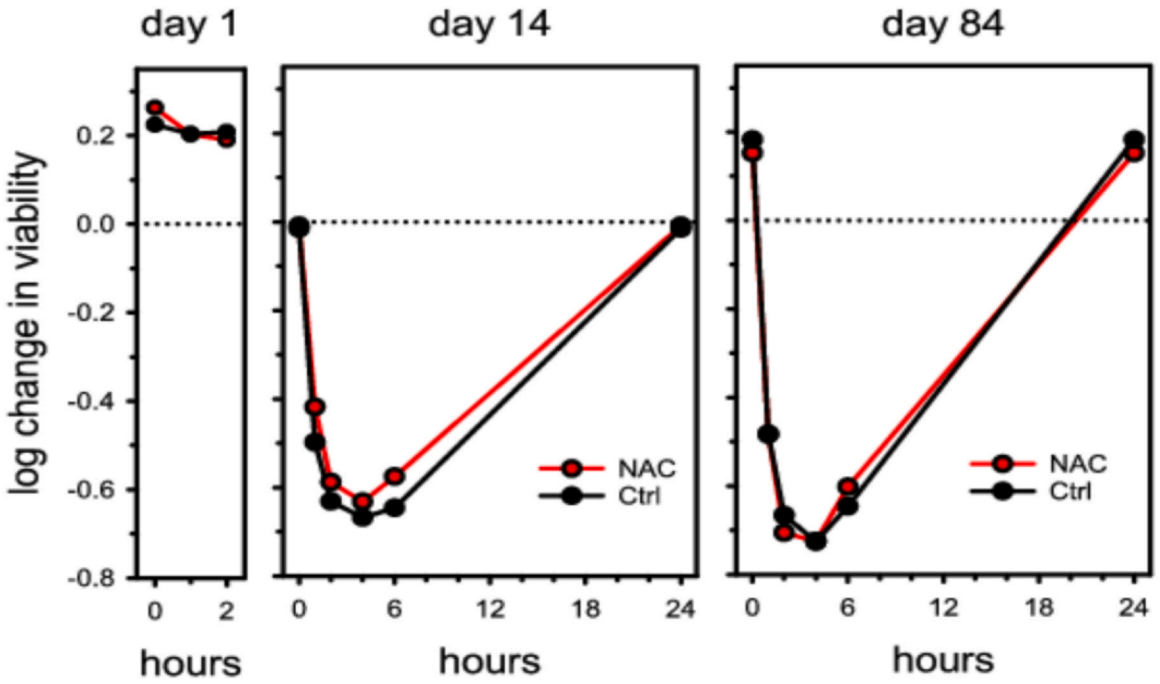

Fig. 4. Mean effects on MTB viability in ex vivo whole blood culture, according to study arm, day, and time post-dose. Positive values indicate growth.

Table 3
Linear mixed effects modeling of NAC effect on MTB survival in ex vivo whole blood culture on study day 1, following the administration of NAC alone to participants.

Source	Estimate	95% CI	P
Intercept	0.22	0.15 – 0.29	< 0.0001
Hours	−0.01	−0.04 – 0.02	0.61
Control arm	0.00		
NAC arm	0.03	−0.07 – 0.14	0.51
Hours* Control arm	0.00		
Hours* NAC arm	−0.03	−0.07 – 0.02	0.22

No effect was evident.

Table 4
Whole blood bactericidal activity (WBA) on days 14 and 84.

	Day 14	Day 84	P
Pre-dose Δlog/day			
NAC	0.00 (−0.09 – 0.05)	0.17 (0.08 – 0.32)	<.001
Ctrl	0.00 (−0.07 – 0.09)	0.22 (0.07 – 0.34)	<.001
P	.83	.543	
AUC0−24hr Δlog			
NAC	−0.38 (−0.44 – 0.24)	−0.32 (−0.37 – −0.25)	.16
Ctrl	−0.38 (−0.48 – −0.32)	−0.31 (−0.43 – −0.18)	.048
P	.29	1.0	

Values indicate median and IQR. Positive values indicate growth. Differences between days 14 and 84, and between NAC and control, were determined by rank sum test. No effect of NAC was evident. The changes from day 14 to day 84 may be attributable to the discontinuation of pyrazinamide and ethambutol.

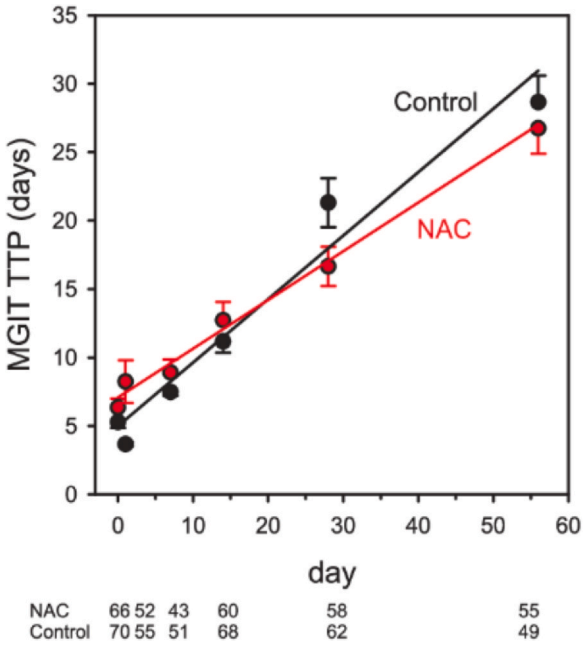

Fig. 5. MGIT TTP according to study day and treatment arm. The lines indicate mixed effects modeling regression. Error bars indicate SEM.

findings of the trial, but also raise additional questions for future research.

Glutathione, the most abundant thiol in mammalian cells, protects against damage by ROS through its oxidation and dimerization: $2GSH+O^{-} \rightarrow GSSG+H_2O$. The reaction consumes glutathione. Cysteine, a conditionally essential sulfur-containing amino acid, is required for glutathione synthesis. Dietary cysteine, found mainly in meat and dairy products, is required to maintain glutathione levels during periods of sustained oxidative stress. The levels of blood cell glutathione we found in healthy Tanzanian volunteers are similar to other healthy populations.[22,23] The strikingly low levels in patients

Table 5
Linear mixed effects modeling of NAC effect on time to positivity in mycobacterial growth indicator tube (MGIT TTP) sputum cultures from baseline to day 56.

Source	Estimate	95% CI	P
Intercept	4.99	3.59 – 6.39	< 0.0001
Day	0.46	0.41 – 0.51	< 0.0001
Control arm	0.00		
NAC arm	2.09	0.08 – 4.11	0.04
Day*Control arm	0.00		
Day*NAC arm	−0.11	−0.18 – −0.04	0.003

Values increased more rapidly in controls compared to NAC recipients, at a rate of 0.11 days of MGIT culture per treatment day.

with tuberculosis likely reflect the combined influence of inflammation, malnutrition, and poverty. The intensity of ROS production in tuberculosis causes newly synthesized glutathione to be rapidly oxidized. This may account for the delay before NAC effects on reduced glutathione become apparent.[12] We report here that total glutathione (the sum of oxidized and reduced) is a rapid, simple, and robust alternative measure of NAC effects in tuberculosis for future clinical trials.

We found clear evidence of the anti-inflammatory effects of NAC using *ex vivo* whole blood culture. As GSH levels rose, MTB-induced TNF production declined in NAC recipients to half of that of controls. There was no corresponding effect on IL-10. These two cytokines reflect the pro- and anti-inflammatory circuits that predominate in tuberculosis.[24] The effect on induced TNF was similar in magnitude to that seen in trials of high dose methylprednisolone and pentoxifylline (an anti-inflammatory phosphodiesterase inhibitor) in Ugandans with HIV and tuberculosis.[25,26] TNF is the likely cause of anemia in tuberculosis, an effect mediated by inhibition of erythropoietin production.[27] The effect of NAC to accelerate the resolution of anemia was similar to that seen with pentoxifylline.[25] These effects are generally consistent with the amelioration of tuberculous immunopathology by NAC seen in preclinical models.[7,8]

Our findings regarding the antimicrobial effects of NAC were less clearcut. NAC has been reported to show direct antimicrobial effects against a wide range of organisms, including mycobacteria, in broth and macrophage cultures[8,28] We collected blood before and shortly after the first NAC dose in this trial to test this observation but were unable to demonstrate any change in intracellular mycobacterial viability due to orally administered NAC. It is possible that the intracellular concentrations achieved after oral NAC dosing are insufficient to show such effects.[15]

Mycobacteria can react to unfavorable external signals (including hypoxia and ROS) by activating a dormancy response in which aerobic respiration and bacillary replication are reduced.[29] Bacilli with this phenotype (sometimes characterized as 'persisters') are less readily killed by most antituberculosis drugs. Vilchèze and Jacobs have proposed that NAC might enhance the antimicrobial activity of certain TB drugs by promoting mycobacterial aerobic respiration and preventing persistence, although they later noted that this phenomenon was highly model dependent.[9,10] Shee *et. al* have reported that NAC specifically enhances the antimycobacterial activity of moxifloxacin,[11] a drug not included in our study. We saw no effect of NAC or GSH on the antimicrobial effects of oral doses of standard TB drugs in *ex vivo* whole blood culture.

The quantitative sputum microbiologic response to tuberculosis treatment has been a subject of interest since the introduction of streptomycin in the late 1940s.[30] Early bactericidal activity (EBA, measured over 1–2 weeks) in these studies appears to mainly reflect drug activity against aerobic, metabolically active extracellular bacilli. EBA trials provide little insight into the ability of a new antituberculosis drug to shorten treatment without increasing the risk of relapse, which appears to arise due to the persistence and subsequent reactivation of semidormant or latent infection. Extending the period of observation to 8 weeks or longer to detect persisters in sputum has been proposed to address this shortcoming.[31] Measuring MGIT TTP would seem well suited to this purpose, as this parameter is inversely related to the log of the inoculum over a wide dynamic range.[32] Moreover, sputum cultures using liquid medium tend to remain positive longer during treatment than those on solid medium, perhaps indicating the recovery of a distinct mycobacterial subpopulation. We included the first negative MGIT result in our analysis (scoring these as 42 days) but censored those occurring subsequently. This approach captures the event of culture conversion without introducing additional statistical complexity. We found that NAC had a small but highly statistically significant effect in delaying the clearance of mycobacteria from sputum during the first 8 weeks of standard TB treatment. It seems this endpoint shows greater statistical power than measures of stable culture conversion, and thus may be more suitable for early phase trials.

However, the ability of either of these microbiologic endpoints to guide regimen development remains uncertain. Extended CFU counting, for example, failed to predict the inability of fluoroquinolone regimens to shorten treatment.[31,33,34] Similarly, measures of stable culture conversion failed to predict that only one of two 4-month rifapentine regimens in study 31 of the TB Trials Consortium would succeed only when moxifloxacin was added.[35] Host biomarkers, either in blood (as gene expression profiles) or lung (as glucose utilization by PET/CT imaging) have been proposed as alternatives. These run the risk, however, that a host-directed adjunctive therapy such as NAC might disrupt the relationship between infection and inflammation, giving misleading results. Phase 2 trials with extended follow-up (as in this trial) may be the most satisfactory alternative in this circumstance.[36]

In summary, adjunctive NAC treatment increased glutathione levels and decreased TNF production in adults with pulmonary tuberculosis. We found no evidence of direct antimicrobial activity of NAC, and divergent effects on two TB treatment biomarkers. Additional studies will be required to determine the effects of adjunctive NAC treatment on microbiologic and pulmonary TB treatment outcomes.

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CRediT authorship contribution statement

DM: Conceptualization, Investigation, Methodology, Writing – original draft preparation. **RSW:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Visualization, Writing – review & editing. **WO:** Formal analysis. **JL:** Investigation. **MK:** Investigation. **MH:** Project administration. **SC:** Project administration. **AR:** Project administration, Resources. **NN:** Resources, Supervision. **BM:** Resources. **IS:** Resources. **ES:** Supervision. **JB:** Supervision. **GC:** Visualization. **AB:** Formal analysis, and **KV:** Supervision. All authors reviewed and approved the manuscript before submission.

Data availability

Anonymized patient level data, relevant dictionaries, and the clinical protocol will be made available from this study at the time of publication through the NIH TB Portals repository.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106379.

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