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TITLE PAGE:

Interferon gamma replacement as salvage therapy in chronic pulmonary aspergillosis; effects on frequency of acute exacerbation and all-cause hospital admission.

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ABSTRACT:

Chronic pulmonary aspergillosis (CPA) is often poorly responsive to antifungal treatment; secondary infections increase morbidity/mortality, particularly in progressive cases. Interferon gamma (IFNγ) has been implicated in not only *Aspergillus* control but also bacterial clearance. Clinical notes of patients with CPA treated with IFNγ (2011-2018) were retrospectively hand-searched. In patients treated for >12 months (n=20), the frequency of acute exacerbation reduced from 3.1 to 1.4 episodes/year (p=0.006) in the 12 months after treatment initiation compared to the 12 months before. A significant reduction in the frequency of hospital admissions/year was also observed (0.8 to 0.3, p=0.04). These findings support further prospective studies.

MAIN TEXT:

Background:

Chronic pulmonary aspergillosis (CPA) is characterised by persistent *Aspergillus* infection, usually complicating pre-existing lung disease such as COPD or bronchiectasis. Although patients are typically without immunocompromise, impaired interferon gamma (IFNγ) and/or interleukin 12 (IL-12) production have been reported.[1] IFNγ prevents conidial germination, enhances alveolar macrophage killing capacity and manipulates Th1 CD4 cell/NK cell influx through chemokine signalling.[2-4] As such, IFNγ supplementation has been explored as a salvage therapy for severe CPA in patients with impaired IFNγ production.[5,6] IFNγ immunotherapy may have additional benefits as CPA is often complicated by recurrent bacterial superinfection; in chronic granulomatous disease, therapeutic IFNγ reduces bacterial infections by up to 70%.[7]

This study reports the impact of subcutaneous IFNγ supplementation on the frequency of acute exacerbation and all-cause hospital admission when administered as salvage therapy to patients with severe CPA, refractory to antifungals, and proven impairment of IFNγ production.

Methods:

For this descriptive study, clinic letters and electronic hospital records were retrospectively hand-searched for all patients prescribed IFNγ salvage therapy for CPA at the National Aspergillosis Centre, Manchester, UK between January 2011 and September 2018 (46 patients). Subcutaneous IFNγ was self-administered at a dose of 50μg three times weekly and prescribed according to clinical judgement when antifungal therapy appeared to be failing due to a combination of clinical, microbiological and radiological parameters in patients with impaired production of IFNγ or IL-12 after *in vitro* cytokine induction.

For the induction of IFNγ, whole blood samples from patients and healthy controls were diluted 1:5 (Roswell Park Memorial Institute medium) into 96 well F plates (Corning) and activated with lipopolysaccharide (List Biochemicals, 1μg/ml) or phytohemagglutinin (Sigma, 10μg/ml), alone or in co-stimulation with IL-12 (Immunotools, 5μg/ml), and incubated at 37°C/5% CO_2. For the induction of IL-12, whole blood was stimulated as above with lipopolysaccharide, alone or in combination with IFNγ (Immukin, Boehringer, $2x10^4$

IU/ml). Supernatants were taken after 24 hours and cytokine levels measured by standard ELISA (IFNγ: Pelikine, Sanquin, NL) or multiplexed particle-based flow cytometry (IL-12: R+D Systems Fluorokinemap), according to the manufacturer's recommendations.

Patients were excluded from analysis if they started IFNγ therapy within 12 months of the patient search (n=5), were lost to follow-up (n=4) or started IFNγ prior to attending clinic (n=1). The frequency of exacerbation, defined as an acute respiratory decline clinically requiring a course of antibiotics, and all-cause hospital admission were compared between the 12 months pre-/post- IFNγ initiation, stratified by duration of IFNγ therapy (Wilcoxon matched-pairs signed-rank test). Incidence rates of death were compared on/off IFNγ for the 36 patients meeting inclusion criteria, starting from the day of IFNγ initiation through to September 2018 for a maximum follow-up of 60 months.

Results:

Of the 36 patients meeting the inclusion criteria, 20 received IFNγ for >12 months, eight stopped treatment due to side effects (all receiving IFNγ for <6 months, mean duration 2.5 months) and eight patients died within 12 months of initiating treatment (mean IFNγ duration 4.6 months, mean survival 7.5 months). Demographic characteristics of these 36 patients are shown in Table 1.

Death rate was statistically similar whilst receiving IFNγ therapy (0.16/year) compared to after stopping IFNγ (0.12/year) (p=0.6), with a median follow-up of 33.5 months (interquartile range 13-45 months). The 28 patients alive at 12 months were included in further analysis.

Twenty-two patients (79%) were on concomitant antifungal therapy and eight (29%) were taking azithromycin long-term whilst receiving IFNγ. Two patients stopped antifungals as they transitioned to IFNγ and azithromycin was started and stopped for one/two patients respectively in the 12 months preceding IFNγ initiation. All remaining patients had the same on/off antifungal and azithromycin status throughout the 24 months of comparison (12 months pre-/post- IFNγ initiation), though most patients on antifungals changed their regimen at some point due to adverse effects, azole resistance and/or clinical failure (Figure 1).

In patients treated for >12 months (n=20), there was a significantly lower number of acute exacerbations and hospital admissions in the year following IFNγ initiation compared to the year prior (Table 2). No significant changes were seen in patients treated with IFNγ for <6 months for both outcomes (n=8). Patients receiving IFNγ for >12 months (versus <6 months) were observed to have a greater difference between pre-/post- IFNγ initiation frequencies of acute exacerbation and hospital admission, although not significantly so for exacerbation. Figure 1 demonstrates the number of acute exacerbations and hospital admissions in the 12 months pre-/post- IFNγ therapy initiation on an individual basis.

Of the 10 patients that remained on the same antifungal treatment throughout the 24 month observation period, eight received IFNγ for >12 months (negative outcomes lower/equal/higher in 6/1/1 patients) and two received IFNγ for <6 months (negative outcomes lower/equal/higher in 0/1/1 patients).

Discussion:

In this retrospective, descriptive study, patients with CPA refractory to antifungals alone appeared to have lower frequencies of acute exacerbation and all-cause hospital admission after the introduction of IFNγ therapy. These effects were observed when IFNγ was given for >12 months, but not for durations <6 months. Our observation is promising as acute exacerbations and hospital admissions are a substantial cause of morbidity and mortality in this patient population.

Almost all patients in our study had impaired IFNγ production and those that did not had reduced levels of IL-12, a cytokine that stimulates IFNγ production. It is not clear if this represents a primary immune defect or a consequence of chronic pulmonary disease or infection. The apparent beneficial effect of IFNγ suggests this is clinically relevant and could be mediated by its antibacterial, antiviral or antifungal action. Due to the chronicity of CPA and the number of patients treated, it was not possible to document a meaningful effect of IFNγ on CPA's clinical course; there was no significant effect on *Aspergillus* serology, extent of CPA on imaging, microbiological eradication of *Aspergillus* or significant change in profile of possible pathogenic organisms from sputum culture (data not shown). The frequency of adverse events and treatment discontinuation was high in this population of severely ill patients.

Long-term antifungals are the treatment of choice for CPA; most of our patients were on azoles. In addition, long-term macrolide treatment is often used for its anti-inflammatory properties and to prevent bacterial exacerbations in patients with chronic lung disease; several of our patients were taking azithromycin during the observation period.[8,9] Admittedly, the concomitant use of antifungal and antibacterial treatment, with changes in regimen occurring in the majority of patients during the 24 month observation, may confound the results of this study. Only a minority of patients, however, had changes in their overall on/off treatment status during this time.

The patients included in this study were highly heterogeneous and treatment was individualised. Given this heterogeneity, using patients as their own comparator was considered to be the fairest way to present this retrospective data but is not without its flaws. Having failed to respond to antifungal therapy they had poor prognosis from the offset. There is potential for introduction for bias if patients with a poorer physiological reserve responded less favourably to IFNγ therapy, self-selecting into the <6 month treatment group. Patients lost to follow-up may also introduce further bias.

Patients that died within 12 months of therapy initiation were not included in the final analysis, arguably introducing bias by excluding the most physiologically vulnerable or patients with the most severe CPA. It is important to note, however, that *all* patients had declining prognostic indicators prior to starting IFNγ. Inclusion of patients that died during the study period would itself have introduced its own bias; the methodology of a self-controlled case series was considered but deemed inappropriate due to death being a common outcome and the number of patients available.[10] There was no evidence of IFNγ therapy being detrimental to health; side effects leading to withdrawal were all mild and there was no difference in death rates between patients on/off IFNγ.

In summary, the frequency of acute exacerbation and hospital admission were lower after the introduction of IFNγ replacement therapy in patients with severe, refractory CPA. Prospective data are needed to further evaluate the role of IFNγ as adjunctive therapy. In addition to control of fungal burden, IFNγ may improve CPA morbidity/mortality by reducing the frequency and severity of bacterial or viral superinfection. These findings could apply to patients with other chronic lung diseases.

Table 1. Baseline characteristics of participants at INFγ testing

Characteristic	Total (n = 36) n (%)
Age group, years	
<40	3 (8)
40-49	1 (3)
50-59	19 (53)
60-69	10 (28)
≥70	3 (8)
Age, years, median [IQR]	56 [51 - 65.5]*
Female sex	18 (50)*
Smoking status	
Active	8 (22)
Ex-smoker	9 (25)
Never	19 (53)
Underlying pulmonary comorbidity†	
ABPA	5 (14)
Asthma	6 (17)
Bronchiectasis	13 (36)
COPD	8 (22)
Fibrosis (secondary to connective tissue disease)	5 (14)
Malignancy	2 (6)
NTM infection (active/previously treated)	6 (17)
Non-COPD bullous lung disease	3 (8)
Pneumothorax (previous)	6 (17)
Sarcoidosis	2 (6)
TB (previously treated)	5 (14)

IFNγ = interferon gamma, IQR = interquartile range, ABPA = allergic bronchopulmonary aspergillosis, COPD = chronic obstructive pulmonary disease, NTM = non-tuberculous mycobacterium, TB = tuberculosis
*Demographics stratified by 12 month survival/exclusion:
Alive (n = 28), median age 56.5 [IQR 50.5-65] years, sex 13/28 (46%) female
Dead (n = 8), median age 55.5 [53-75 IQR] years, sex 5/8 (63%) female
†Not mutually exclusive: patients could have more than one underlying pulmonary comorbidity

Table 2. Number of acute exacerbations and hospital admissions pre-/post- IFNγ initiation

Outcome and population	Mean events over 12 months (standard deviation)		P value
	Pre- IFNγ initiation	Post- IFNγ initiation	
Acute exacerbations (clinically requiring antibiotics)[a]			
IFNγ duration			
>12 months (n = 20)	3.1 (2.4)	1.4 (1.5)	0.006
<6 months (n = 8)	2.8 (1.8)	2.6 (1.8)	0.7
Hospital admissions[a]			
IFNγ duration			
>12 months (n = 20)	0.8 (0.9)	0.3 (0.6)	0.04
<6 months (n = 8)	0.6 (0.7)	1.0 (1.1)	0.5
Comparison between >12 and <6 month IFNγ duration[b]			
Acute exacerbations	-	-	0.06
Hospital Admission	-	-	0.02

[a] Wilcoxon matched-pairs signed rank test, [b] t-test
IFNγ = interferon gamma

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Contributions to authorship:

CK, conceived the project; RD coordinated and interpreted immunodeficiency testing; CH and CK performed the patient search; EJMM and CK performed data collection; EJMM and CK analysed and interpreted the data; EJMM and CK drafted the manuscript; CH, RD, GH, and DWD critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Potential conflicts of interest:

Professor Denning and family hold Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company. He acts or has recently acted as a consultant to Scynexis, Cidara, Pulmatrix, Zambon, iCo Therapeutics, Roivant and Fujifilm. In the last three years, he has been paid for talks on behalf of Dynamiker, Hikma, Gilead, Merck, Mylan and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group and the British Society for Medical Mycology Standards of Care committee. EJMM, CH, RD, GH and CK have no conflicts of interest to declare.

References:

1. Döffinger R, Harris C, Lear S, et al. Reduced gamma interferon (gIFN) production in chronic pulmonary aspergillosis (CPA). Abstract 96, 5th Advances Against Aspergillus Conference, 2012. Available at: https://www.aspergillus.org.uk/content/reduced-gamma-interferon-gifn-production-chronic-pulmonary-aspergillosis-cpa [accessed May 2019].
2. Bouzani M, Ok M, McCormick A, et al. Human NK cells display important antifungal activity against Aspergillus fumigatus, which is directly mediated by IFN-γ release. *J Immunol.* 2011;Aug 1;187(3):1369-76 doi: 10.4049/jimmunol.1003593 [published Online First: 22 June 2011].
3. Park SJ, Hughes MA, Burdick M, et al. Early NK cell-derived IFN-{gamma} is essential to host defense in neutropenic invasive aspergillosis. *J Immunol.* 2009;Apr 1;182(7):4306-12 doi:10.4049/jimmunol.0803462.
4. Pak-Wittel MA, Yang L, Sojka DK, et al. Interferon-γ mediates chemokine-dependent recruitment of natural killer cells during viral infection. *Proc Natl Acad Sci U S A.* 2013;Jan 2;110(1):E50–E59 doi: 10.1073/pnas.1220456110 [published Online First: 17 December 2012].
5. Denning DW, Cadranel J, Beigelman-Aubry C, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. *Eur Respir J.* 2016;Jan;47(1):45-68 doi: 10.1183/13993003.00583-2015.
6. Kosmidis C. and Muldoon EG. Challenges in the management of chronic pulmonary aspergillosis. *Med Mycol.* 2017;Jan 1;55(1):63-68 doi: 10.1093/mmy/myw119 [published Online First: 2 December 2016].
7. Errante PR, Frazao JB and Condino-Neto A. The use of interferon-gamma therapy in chronic granulomatous disease. *Recent Pat Antiinfect Drug Discov.* 2008;Nov;3(3):225-30 doi: 10.2174/157489108786242378.
8. Li W, Qin Z, Gao J, et al. Azithromycin or erythromycin? Macrolides for non-cystic fibrosis bronchiectasis in adults: A systematic review and adjusted indirect treatment comparison. *Chron Respir Dis.* 2019;Jan-Dec;16:1479972318790269 doi: 10.1177/1479972318790269 [published Online First: 12 August 2018].
9. Cui Y, Luo L, Li C, et al. Long-term macrolide treatment for the prevention of acute exacerbations in COPD: a systematic review and meta-analysis. *Int J Chron Obstruct Pulmon Dis.* 2018;Nov 22;13:3813-3829 doi: 10.2147/COPD.S181246.

10. Petersen I, Douglas I and Whitaker H. Self-controlled case series methods: an alternative to standard epidemiological study designs. *BMJ.* 2016;Sep 12;354:i4515 doi: 10.1136/bmj.i4515.

Figure legend:

Figure 1.

Individual and mean frequencies of acute exacerbation and hospital admission in the 12 months pre-/post- IFNγ initiation, with illustration of concomitant antifungal/azithromycin treatment regimen, stratified by IFNγ treatment duration. SD = standard deviation.