Impact of liposomal amphotericin B therapy on chronic pulmonary aspergillosis

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Title:

Impact of liposomal amphotericin B therapy on chronic pulmonary aspergillosis

Running title:

Liposomal amphotericin B in CPA

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Abstract

Objectives: To assess the clinical response and renal toxicity observed in chronic pulmonary aspergillosis (CPA) patients receiving ≥1 short-courses of liposomal amphotericin (LAmB) (AmBisome®) therapy.

Methods: A retrospective audit of clinical response and renal function was undertaken in 71 CPA patients (41 male) treated with LAmB at the National Aspergillosis Centre, including 20 patients receiving repeated treatment courses or long-term therapy (n=5).

Results: Median age was 64 years (range 29 – 86 years). Treatment indications included respiratory symptoms (N=33; 46.5%), constitutional symptoms (N=2; 2.8%) or both (N=36; 50.7%). 48 patients (73.8%) responded to their first LAmB course. Quality of life (QOL) improvements occurred in 37 (92.5%) of 40 patients with sufficient data available. Response rates for repeated short-courses of LAmB were 76.6%; QOL improvements were observed in 91.7% of treatment courses. All patients on long-term therapy demonstrated a response. 34 (50%) and 17 (25%) patients respectively developed an increased risk of acute kidney injury (AKI) or actual AKI with their first treatment; a significant reduction in geometric mean eGFR was observed and a similar pattern occurred following their second treatment course.

Conclusions: Whilst CPA is responsive to LAmB, caution should be exercised with repeated courses, if other treatments are available.

Key words:

Highlights:

- Clinical response to the first LAmB course in CPA patients was 74%
- Improvements in QOL in CPA patients after their first LAmB was 92%
- 50% of CPA patients had an increased risk of AKI with their first LAmB course
- 25% of CPA patients developed AKI with their first LAmB course
- The geometric mean eGFR in CPA patients was reduced post LAmB therapy

Introduction

Intravenous amphotericin B therapy was first used for chronic pulmonary aspergillosis (CPA) in 1959, the first reported case of aspergillosis ever treated with amphotericin B [1]. A few subsequent reports have documented efficacy in a small number of patients, mostly treated with deoxycholate amphotericin B [2,3]. In contrast, there is extensive experience of amphotericin B for invasive pulmonary aspergillosis, including some randomised controlled trials [4-10] that show equivalent efficacy between different amphotericin B preparations with reduced toxicity of liposomal amphotericin B in most patients.

There are some specific potential advantages for amphotericin B for treating CPA. First, almost all the cases are caused by *Aspergillus fumigatus*. *A. fumigatus* is very rarely overtly resistant to amphotericin B unlike *Aspergillus nidulans* and *Aspergillus terreus* which are intrinsically resistant to amphotericin B (and may cause invasive aspergillosis) [11-15]. Second, triazole resistance is an increasing problem in *A. fumigatus* in many countries, especially northern Europe [16-20] and so intravenous therapy with either amphotericin B or an echinocandin are appropriate treatment options [21]. Third, CPA patients have subtle, poorly defined immunodeficiency [22-24] which may benefit from up-regulation of TH1 responses, which specifically liposomal amphotericin B does more so than deoxycholate amphotericin B [25].

Our prior small experience of treating CPA with deoxycholate amphotericin B was positive in terms of efficacy [2] but carried a major toxicity burden with 64% of patients having some significant adverse event over 3 weeks of therapy, if that length of therapy could even be delivered. About 5 years ago, we switched to liposomal amphotericin B (AmBisome) (LAmB) for all patients, with fewer toxicity issues. Here we report our clinical outcomes and adverse events with LAmB used to treat CPA.

CPA is usually a long-term progressive illness, causing either or both respiratory and systemic symptoms. As a result, different strategic approaches can be considered when using amphotericin therapy. The first approach is simply an initial, 'induction' course of therapy in newly diagnosed, but very ill patients with CPA. The logic for its use as initial therapy is to ensure IV treatment as opposed to oral delivery, to provide some 'immune stimulation' with upregulation of TH1 responses and to circumvent any concerns about triazole resistance. In patients with pan-azole resistant infection, a switch to intravenous echinocandin or amphotericin B is necessary, if any useful antifungal therapy is to be delivered. Such therapy

could be delivered as occasional intravenous courses of therapy, or as continuous therapy. We have used both these longer term strategies and report both here.

Patients and methods

Study design and data collection

This audit was a retrospective case-note review of patients with CPA who received at least one short course of LAmB and any subsequent courses of treatment, under the care of the National Aspergillosis Centre (NAC) in Manchester, UK. CPA was defined using the definition described by Farid et al [26] and is consistent with the definition provided in the latest European Society for Clinical Microbiology and Infectious Diseases and European Respiratory Society guidelines [27]. Patients were eligible for inclusion in the audit if they had received one or more short courses of LAmB treatment, or a short course of LAmB treatment followed by intermittent long-term LAmB treatment, before July 2013. A short course of LAmB treatment was defined as daily treatment of less than six weeks in duration whilst intermittent long-term treatment was treatment given three to four times a week for at least 2 months duration. Intermittent long-term treatment was given for at least 2 months duration until the patient either did not require further antifungal therapy, clinically failed treatment or developed significant toxicities associated with their treatment. Intravenous LAmB was made up in normal saline and protected from the light. All patients received an initial test dose of 1 mg of LAmB intravenously given over 10 – 15 minutes and if tolerated they received their treatment dose over 2 – 3.5 hours.

A standardised proforma was used for data collection. Details recorded included patient demographics, co-existing respiratory illnesses in addition to their CPA, the main clinical indications for LAmB treatment including respiratory (cough, haemoptysis, shortness of breath including their MRC dyspnoea score if known ; n=3 symptoms) and constitutional symptoms (weight loss, appetite, lethargy and fatigue, reported worsening quality of life (QOL); n = 4 symptoms), the use of intravenous fluid rehydration prior to commencing LAmB treatment, the use of amiloride or other diuretics, the dose and duration of LAmB therapy and whether dose modifications were made for renal toxicity reasons. Their pre-treatment eGFR (estimated glomerular filtration rate), the lowest eGFR on LAmB treatment and their eGFR results at 1 month, 3 to 4 months and 6 to 8 months after treatment completion were recorded. Estimated eGFR measurements were calculated using the renal calculator found at **www.egfrcacl.renal.org/.** The risk of or development of an acute kidney injury (AKI) was defined using the RIFLE criteria where abrupt changes in renal function

were of at least 24 hours in duration over 1 to 7 days [28]. Risk of AKI was defined as a decrease in eGFR of > 25% and injury occurred when the eGFR had fallen by > 50%. Assessment of the risk and development of AKI was performed for all patients receiving their first course of LAmB treatment; this occurred for the duration of their therapy and for the following 7 days after their last dose of treatment. Information was collected on all potentially nephrotoxic medications and intravenous contrast media (for CT scan or bronchial artery embolization) given around the time of LAmB therapy. The development of chronic kidney disease (CKD) post LAmB therapy was defined using the categories of chronic kidney disease described in the KDIGO 2012, based on their eGFR [29]. The response to therapy was assessed in the 6 months following treatment and included collecting data on improvements in their respiratory and constitutional symptoms, their immunological and mycological response to treatment (if available) and their St George's respiratory Questionnaire QOL scores pre- and post-treatment (if available) [30].

Treatment response in the six months after LAmB therapy

Treatment responses were assessed in all patients who had received a minimum of three doses of LAmB therapy. A clinical response to treatment was defined as an improvement in at least two symptoms from the same symptom group; a 2 Kg weight gain was used as the criteria for improvement in patients experiencing weight loss. Stable weight was defined as a change of <2 Kg in weight pre- and post-treatment and weight loss was defined a losing >2 Kg in weight. Improvements in haemoptysis were excluded from analysis if the patient had undergone bronchial artery embolization during LAmB therapy. An immunological response to treatment was defined as a four-fold drop in the *Aspergillus* precipitins antibody titre (Microgen, Camberley, UK) or a fall of > 50 mg/L in the *Aspergillus* specific IgG antibody titre (Phadia, Uppsala, Sweden). The mycological response was assessed in patients who had a positive sputum *Aspergillus* culture or *Aspergillus* PCR result prior to LAmB treatment. Fungal DNA extraction using MycXtra™ (Myconostica, Camberley, UK) and a commercial real time PCR assay, (MycAssay™ Aspergillus, Myconostica), which targets a portion of the 18S ribosomal gene were done in the Mycology Reference Centre, Manchester. The failure to detect *Aspergillu*s in their sputum, using the same methodology as their pre-treatment sample, post LAmB therapy was used to define a mycological response. Improvements in QOL were defined as an improvement of ≥4 points in at least one modality (symptom, impact, activity, total) in the St George's QOL score [30]. A stable QOL score was defined as a change in score of less than 4 points in either direction whilst a deterioration was defined as an increase of ≥4 points.

All patients who received at least one full dose of LAmB therapy had their renal function assessed pre- and post-treatment. Patients who developed an AKI on LAmB therapy were given daily intravenous fluid (normal saline) rehydration and any concomitant nephrotoxic drugs stopped. Their renal function was closely monitored and in some instances the dose of LAmB given was reduced. The decision to dose reduce therapy and / or stop LAmB therapy was made by the treating physician on an individual basis although there was a general policy to stop treatment in the event that a patients' eGFR fell to 40 ml/min/ 1.73m^2.

Statistics

Categorical data was reported as total numbers and percentages. Normally distributed and non-normally distributed data was reported as mean or median values respectively. Creatinine and eGFR data was $\log_{10}$ transformed prior to analysis to enable approximation to a normal distribution. A longitudinal regression analysis with linear response using generalised estimating equations with an autoregressive correlation structure was carried out to assess changes in creatinine and eGFR over time. For assessing the proportion of eGFR values below 90, a binary logistic response was used in the regression model.

Results

Seventy one CPA patients (41 male, 30 female) were treated with LAmB therapy, of whom 20 patients had received 2 or more short courses and 5 patients had received at least one short course of treatment followed by intermittent long-term therapy. Their age ranged between 29 and 86 years (median 64 years) at the time of their first dose of LAmB therapy. Their baseline demographics, including underlying respiratory conditions apart from CPA and baseline renal function, is provided in **Table 1**. All but two patients had other underlying respiratory conditions, the commonest being a history of _Mycobacterium tuberculosis_ or a non-tuberculous mycobacterial infection, chronic pulmonary obstructive disease, bronchiectasis or interstitial lung disease. Three patients had received between one and three courses of intravenous amphotericin B therapy (each patient had received one course of LAmB) at their local hospital, prior to referral to the NAC. The median duration of prior azole therapy at the time of their first LAmB course was 12 months with 50 (70.4%) patients having received two or more azoles in the past. Nineteen patients (26.7%) had _A. fumigatus_ in their sputum that was resistant to one or more azoles at the time of their first course of LAmB therapy. Ten (14.1%) patients had infection with a pan-azole resistant _A. fumigatus._ The median duration of follow-up following their first course of LAmB therapy was 22 months (range 0 – 92

months; n=69 patients). At the time this paper was written 35 (43.5%) of the 69 patients who received at least 3 doses of LAmB were still alive.

Clinical indication and treatment response to the first short course of LAmB therapy

The primary indication for treatment was either respiratory symptoms (N= 33, 46.5%; mean number of symptoms was 2.34), constitutional symptoms (N=2, 2.8%); mean number of symptoms was 2.17) or both (N= 36; 50.7%; mean number of symptoms 4.51). Two patients received treatment based primarily on their constitutional symptoms of weight loss and severe lethargy and fatigue.

Twenty seven (38%) patients received intravenous fluid rehydration prior to their first few doses of LAmB therapy. The daily dose of LAmB given ranged between 2.5 – 5.0 mg/kg/day (median 3.0 mg/kg/day). Two patients (2.8%) were unable to tolerate the test dose of LAmB therapy and did not receive any further treatment. One additional patient suffered an acute fall in their eGFR by 35% within two days of starting therapy and did not receive any further treatment. This patient had not received IV fluid rehydration prior to their first dose of LAmB. The duration of therapy for the remaining patients (n=68; 95.8%) ranged between 4 to 36 days (median 20.5 days).

The response to treatment was assessed in 65 patients. Six patients were excluded from the analysis; two patients were unable to tolerate LAmB, one patient had an acute drop to their eGFR and three patients started intermittent long-term LAmB treatment within one month of completing their first short course of LAmB therapy. Overall 48 patients (73.8%) had a clinical response to treatment, as shown in **Table 2.** Improvements in respiratory symptoms were noted in 39 patients (60%); 22 of 29 (75.9%) patients with pre- and post-treatment MRC dyspnoea scores experienced at least a 1 point improvement in their score. Thirty patients (46.2%) noticed improvements in their constitutional symptoms with 27 (41.5%) experiencing at least a 2 Kg weight gain and 9 (13.8%) gaining > 5 Kg in weight. Improvements in both respiratory and constitutional symptoms were seen in 21 patients (32.3%) whilst 27 (41.5%) had a response in either their respiratory or constitutional symptoms. Seventeen patients (26.1%) did not improve in either their respiratory or constitutional symptoms.

Sufficient data was available to assess the immunological response to treatment in 48 patients (73.8%), 12 of whom (25%) experienced a response to treatment. Seven patients had *A. fumigatus* cultured in their sputum just prior to LAmB therapy and following treatment the

culture was negative in 2 patients (28.6%). Thirteen patients had a positive sputum *Aspergillus* PCR pre-treatment and post-treatment the *Aspergillus* PCR was negative in 8 patients (61.5%).

SGRQ QOL data was available in 40 patients pre- and post-treatment and 37 patients (92.5%) noticed an improvement of 4 or more points in at least one modality. Improvements in the symptom, impact, activity and total scores are shown in **Figure 1**. A major 12 point improvement in each modality was seen in at least 30% of patients.

The clinical response to LAmB therapy was often delayed and only noticeable following completion of their LAmB treatment course. Thirty seven patients who responded to LAmB therapy did not receive an oral azole following their treatment course. The duration of their clinical response and remission ranged between 0 – 25 months (median 4 months) after completing treatment; two patients started to deteriorate within 2 – 3 weeks of finishing LAmB therapy.

Clinical indication and treatment response to repeated short courses of LAmB therapy

Twenty patients received two or more short-courses of LAmB therapy (2 courses, n=14; 3 courses, n=5; 4 courses, n=1; total number of courses given was 47). One patient received a short course of treatment, then intermittent long-term treatment followed by three further short courses of LAmB therapy; their intermittent long-term treatment had been stopped due to concerns about early clinical failure and renal toxicity. The median interval between each short course of treatment was 6 months (range 2 – 67 months, excluding the patient who received intermittent long-term treatment between their other short courses of treatment).The median duration of prior azole therapy at the time of their first course of LAmB therapy was 20.5 months (range 1 to 74 months) and 17 patients (85%) had received prior treatment with two or more azoles.

The main indication for each short course of treatment (n=47 courses of treatment) was either respiratory symptoms (N= 24, 51.1%; mean number of symptoms was 2.34) or both respiratory and constitutional symptoms (N= 23; 48.9%; mean number of symptoms 4.55). Eleven (55%) of 20 patients had the same clinical indication for each course of treatment they received.

The daily dose of LAmB given ranged between 2.2 – 3.83 mg/kg/day (median 2.87 mg/kg/day). The overall clinical response rate for the 47 short courses of LAmB given was

76.6% as shown in **Table 3.** Six (30%) of 20 patients did not experience a clinical response to their first course of LAmB treatment but all six responded to a subsequent short course of treatment.

Improvements in respiratory symptoms were observed with 26 (55.3%) courses of treatment; 10 of these courses (38.5%) were associated with an improvement of at least 1 point in the patients' MRC dyspnoea score following treatment. Twenty four treatment courses (51.1%) were associated with a response in constitutional symptoms; a weight gain of at least two kilograms was seen with 21 (44.7%) courses of treatment and weight gain of over 5 Kg was seen with 5 (10.6%) treatment courses. **Figure 2** illustrates the variable weight response observed between treatment courses for 13 patients who had at least 2 courses of LAmB treatment (courses received = 33). The figure demonstrates their initial weight response to the first course of treatment and whether a similar response was observed with subsequent courses of treatment. Two patients lost weight with both treatment courses and in one patient his weight was stable with each treatment course. Five patients gained weight with each course of treatment whilst 8 patients had a variable weight response to the treatment courses they received.

Sufficient immunological data was available to assess the immunological response for 42 (89.4%) of the 47 short courses of LAmB treatment. Nine patients (45%) experienced an immunological response to treatment (n=13 courses) two of whom responded to both short courses of therapy. Five patients had a positive sputum culture for *Aspergillus fumigatus* prior to at least one LAmB course (n=7 courses of treatment) and following therapy all patients had a negative culture (n=5 courses of treatment). Seven patients had a positive *Aspergillus* PCR in at least one pre-treatment sputum sample (n=11 courses of treatment) and following treatment the sputum *Aspergillus* PCR was negative in five patients (n=6 courses of treatment; 54.5% of the treatment courses).

SGRQ QOL scores pre- and post-LAmB therapy were available for 36 courses of treatment (n=17 patients) and a response in one or more modalities was observed with 33 (91.7%) treatment courses. There were improvements in the symptom, impact, activity and total scores for 19 (52.8%), 22 (61.1%), 18 (50.0%) and 21 (58.3%) courses respectively; a major 12 point improvement was observed in these modalities in 14 (38.9%), 15 (41.7%), 16 (44.4%) and 13 (36.1%) treatment courses respectively. Thirteen patients had SGRQ QOL scores available pre- and post-treatment for at least two courses of treatment (n=34 courses). The variation in their QOL responses is shown in **Figure 3.** The majority of patients had a variable response in their QOL scores between treatment courses; a smaller proportion of patients

showed a consistent improvement in one or more modalities of their SGRQ QOL score with repeated short courses of treatment.

The treatment response to intermittent long-term LAmB therapy

Five patients started long-term LAmB therapy within a month of completing a short course of treatment (3 patients after their first short course of therapy and 2 patients after their second short course of treatment). The main clinical indication for treatment was respiratory symptoms (n=1), constitutional symptoms (n=2) or both respiratory and constitutional symptoms (n=2). Intermittent long-term treatment was generally given to patients who had no oral azole treatment options available due to either intolerance and /or resistance apart from one patient who had a major deterioration on itraconazole prior to receiving LAmB. This patient later tried voriconazole but was intolerant of it.

The dose of LAmB given as long-term treatment three times a week ranged between 2.53 – 6.7 mg/Kg/dose (mean 4 mg/kg/ dose); the highest dose of 6.7 mg/Kg/dose was given to a patient who had a multi-azole resistant *Aspergillus fumigatus* infection that had not been controlled on a dose of 5.4 mg/kg/dose of LAmB given three times a week. The duration of treatment ranged between 4 and 24 months (median 11 months).

All five patients showed a clinical response to treatment. Four patients had an improvement in their chest symptoms and three patients noticed an improvement in their constitutional symptoms. The mean number of symptoms that improved was 3.8 (range 3 – 5 symptoms) and two (40%) patients experienced an immunological response to treatment. Four patients later failed LAmB and one patient stopped long-term therapy following a completion pneumonectomy. Two patients subsequently died with CPA and end stage respiratory disease.

Acute kidney injury associated with LAmB therapy

The majority of patients had either normal renal function (grade 1 CKD; n=48, 67.7%) or mild renal impairment (grade 2 CKD; n=20, 28.2%) prior to their first course of LAmB therapy. There were three (4.2%) patients who had stage 3a CKD prior to treatment, as shown in **Table 1**.

a) Renal function following first short course of LAmB therapy

Changes in eGFR were commonly seen in patients receiving their first short course of LAmB therapy. Fifty one of 68 (75%) patients experienced a fall of over 25% in their eGFR during their first course of therapy, resulting in an increased risk of AKI, of whom 17 (25%) patients developed an AKI with treatment. One patient was excluded from the analysis as he had short courses of treatment before and following intermittent long-term LAmB therapy. **Figures 4A and 4B** illustrates the proportion of patients who developed an increased risk of AKI or AKI during their first course of LAmB therapy; the largest proportion of patients had a fall of >25% in their eGFR during their second week of treatment. The use of intravenous fluid rehydration therapy prior to the first few doses of LAmB therapy did not reduce the proportion of patients who developed increased risk of AKI or an AKI; 7 (25.9%) out of 27 patients with intravenous rehydration maintained their renal function throughout their course of treatment compared to 11 (26.8%) of 41 patients who did not receive pre-treatment intravenous fluid rehydration.

Twenty four (35.3%) of the 68 patients treated with LAmB had at least one other contributing factor to the development of an increased risk of AKI or an AKI. Seventeen (25%) patients had received intravenous contrast and of these patients 9 (52.9%) developed an increased risk of AKI and 5 (29.4%) an AKI. Nine (13.2%) of 68 patients were receiving other concomitant medications with associated nephrotoxicity risks (NSAIDs, n=1; polymyxin, n=1; acyclovir, n=1; aminoglycosides, n=6) and all these individuals experienced either an increased risk of AKI (n=6) or an AKI (n=3). In total 8 (15.7%) of 51 patients with either an increased risk of AKI or AKI stopped their LAmB therapy early, despite 4 having a LAmB dose reduction prior to stopping treatment.

An overall deterioration in renal function was observed in patients during and following their first course of LAmB therapy, as shown in **Figures 5A and Figure 5B.** The adjusted geometric mean eGFR fell during treatment and did improve post treatment but did not return to pre-treatment levels ($p < 0.001$). The adjusted percentage of individuals with an eGFR < 90 increased following treatment and this was also statistically significant ($p < 0.001$).

b) Renal function following the second short course of LAmB therapy

Nineteen patients had their renal function assessed prior to and following their second course of treatment. **Figures 6A and 6B** show the adjusted geometric mean eGFR and adjusted percentage of individuals with an eGFR < 90 during and following their second course of

LAmB therapy (n=19). There was a statistically significant decline in the adjusted geometric mean eGFR over the time-frame illustrated (p < 0.001) but no change in the adjusted percentage of individuals with an eGFR < 90 following treatment (p = 0.57). On reviewing their renal function prior to their first course of LAmB therapy there were 11 patients with normal renal function (grade 1 CKD), 7 patients with mild renal impairment (grade 2 CKD) and 1 patient with grade 3 CKD. Following their second course of treatment 8, 9 and 2 patients respectively had grade 1, grade 2 and grade 3 CKD indicating that a proportion of patients had developed worsening CKD following their two courses of LAmB therapy.

c) Renal function with long-term interment LAmB therapy

Five patients had intermittent long-term LAmB therapy, three of whom had stable renal function throughout treatment. Two patients stopped treatment due to worsening renal function. One patient developed Stage 4 CKD and nephrogenic diabetes and the remaining patient had deteriorating renal function (Stage 3A to Stage 3B) despite a dose reduction in LAmB.

Conclusion

In last 40 years, amphotericin B has been frequently used for the treatment of invasive aspergillosis (IA) with modest outcomes [31]. Pulmonary localization of infection carried a medium likelihood of survival compared with brain or sinus infection, with an overall survival rate of 14% in collected series [31]. In a small prospective randomized study completed in 1991, 28 neutropenic patients with IA were given deoxycholate amphotericin B with or without flucytosine, only 5 survived (17.9%) [4]. The relatively low response and survival rates showed some improvement in a prospective randomized controlled study comparing deoxycholate and colloidal dispersion formulations completed in 1997 [9]. Highly immunocompromised patients including 42% allogeneic hematopoetic stem cell transplant recipients enrolled without antigen, PCR or CT scanning criteria alone (and therefore often late diagnoses) had a 13.8% response rate and a 47.3% 12 week survival rate. The registration studies for voriconazole utilized deoxycholate amphotericin B, and found an overall survival rate of 57.9% at 12 weeks, with 80.5% of patients switching to alternative therapy, usually a lipid formulation of amphotericin B in those commenced on deoxycholate amphotericin B [8]. In the 26 patients who only received deoxycholate amphotericin B, 1 (4%) responded to therapy [32]. This randomised study enrolled haematology patients on the basis of CT scan appearances only, without antigen detection. A later study almost exclusively in the 'good' prognostic group of acute leukaemia utilized both CT scanning and antigen detection for

enrolment [10]. Here 65.7% of patients survived to 12 weeks, considerably better than in prior studies, probably primarily because of earlier diagnosis, lower intensity hematopoetic stem cell transplantation and neutrophil recovery. This trend towards improved outcomes has also been noted in large collected series with typically ~45% of patients treated with any amphotericin B formulation surviving to 12 weeks [33].

The situation in CPA is different because patients are not immunocompromised and there is no expectation of an overt change in immune status during treatment, as with neutrophil recovery. Therefore one might expect improved response rates. However in IA treatment studies, the key determinants of outcome are survival and radiographic improvement, neither of which pertain to CPA in a 3 week time frame of amphotericin B therapy. So therapeutic response is assessed on the basis of a quality of life scores, weight gain, general symptoms and downward trends in *Aspergillus* antibody titres and inflammatory markers and sputum culture or PCR conversion to negative. Here, 75% of those treated with LAmB improved clinically, to variable degrees, with both their first and subsequent treatment courses. This response rate is similar to those treated with deoxycholate amphotericin B (82% improved or stable) [2]. All patients on long-term intermittent LAmB demonstrated a clinical response.

Several studies have demonstrated that LAmB has less nephrotoxic effects than conventional amphotericin B in the presence or absence of other concomitant nephrotoxic drugs [9, 34,35]. The proportion of patients experiencing significant nephrotoxicity during treatment with intravenous amphotericin B (conventional or liposomal) increased with the addition of one or more concomitant nephrotoxic medications [34, 36]. These studies defined significant nephrotoxicity as either reaching an absolute peak serum creatinine level of 265umol/L [34] or a doubling or tripling of baseline serum creatinine levels during LAmB therapy [34-36]. Walsh et al [34] described significant nephrotoxicity in 33.7% of patients receiving 0.6 mg/Kg/day of conventional amphotericin B compared to 18.7% of patients treated with 3 mg/kg/day of LAmB. In the study of Prentice et al [35] in leukaemia patients, nephrotoxicity was observed in 24% of patients on 1mg/Kg/day of conventional amphotericin B compared to 12% of patients receiving 3mg/Kg/day of LAmB. The different liposomal preparations of amphotericin B have been shown by Wingard et al [36] to have differing nephrotoxicity rates; treatment with 3mg/Kg/day or 5mg/Kg/day of LAmB was associated with nephrotoxicity in 14% of patients compared to 42% of patients treated with amphotericin B lipid complex at a dose of 5 mg /kg/day. If we had used a definition of significant nephrotoxicity of a peak serum creatinine level of 265umol/L, then no patients would be defined as having significant

nephrotoxicity with one or more treatment courses with 3mg/Kg/day LAmB or only 17.4% if a doubling of serum creatinine was used to define nephrotoxicity.

The estimated glomerular filtration rate is now used as the main tool to assess renal function, both in AKI and CKD. Deterioration in renal function was commonly seen during LAmB therapy whether or not patients received pre-rehydration with intravenous fluids. Fifty one of 68 (75%) patients experienced a fall in their eGFR of over 25% during their first course of therapy resulting in an increased risk of AKI, according to the RIFLE criteria [28], in 34 (50%) patients and an AKI in 17 (25%) patients. Twenty four (35.3%) of 68 patients had at least one other contributing factor to the development of an increased risk of AKI or an AKI; 17 (25%) patients had a contrast scan and 9 (13.2%) patients were receiving potentially nephrotoxic medications. Only 3 (12.5%) of 24 patients with an additional potential nephrotoxicity risk avoided an increased risk of AKI or AKI during their LAmB treatment. The deterioration in renal function observed during and following the first course of LAmB therapy was statistically significant; both the geometric mean eGFR fell and the adjusted percentage of individuals with an eGFR <90 (equivalent to CKD Stage 2 or more) significantly increased (both p values were <0.001). A similar deterioration in the adjusted geometric mean eGFR was observed for the 19 patients who received a second short course of LAmB therapy (p <0.001) although there was no significant change in the percentage of individuals with an eGFR < 90 (p = 0.57). There was a higher proportion of patients with grade 2 and grade 3 CKD after their second course of LAmB treatment.

Avoidance of intravenous contrast (for CT scans or bronchial artery embolization) or other medications with known associated nephrotoxicity is prudent during LAmB therapy. N-acetylcysteine may ameliorate nephrotoxicity as shown by Karimzadeh et al [37] in a double blind placebo controlled study (p =0.049), at the cost of increased other adverse reactions.

Here we document that repeated short courses of LAmB therapy are associated with deterioration in renal function, as determined by a fall in the adjusted geometric mean eGFR following treatment. It raises the question of whether repeated courses of LAmB to control CPA disease should be avoided, if other intravenous therapies such as echinocandins are a treatment option. However the response rate to LAmB was generally good, with clinical benefit of some level in most patients. Comparative studies with other agents are necessary to truly compare response and toxicity, but this large retrospective series sets the stage for such studies by providing effect estimates that can be used for estimating study size for different endpoints.

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Table 1
Baseline demographics and patient characteristics at the time they received their first course of IV LAmB therapy.

Variable	Number (% or median)
Sex (M) (%)	41 (57.7%)
Age (years)	29 – 86 (median 64)
Underlying respiratory disease	
Mycobacterial infection (TB/NTM)	20 (28.2%)
COPD	20 (28.2%)
ABPA with Asthma	9 (12.7%)
Asthma with no ABPA	4 (5.6%)
Bronchiectasis	15 (21.1%)
Previous pneumothorax	9 (12.7%)
Previous thoracic surgery	6 (8.4%)
Lung cancer	4 (5.6%)
Previous thoracic radiotherapy	4 (5.6%)
Interstitial lung disease	13 (18.3%)
Other*	6 (8.4%)
None	2 (2.8%)
Duration of previous azole therapy (n=71 patients)	0 – 112 months (median 12)
Itraconazole (N= 62 patients)	1 – 67 months (median 7)
Voriconazole (N= 53 patients)	1 – 55 months (median 6.5)
Posaconazole (N=29 patients)	1 – 43 months (median 11)
Main indication for discontinuing prior azole therapy	

Itraconazole (N=62 patients)
  Intolerance and / or toxicity
  Clinical failure and / or documented resistance
	N=30 (48.4 %)
	N=35 (56.4 %); documented resistance was seen in 19 (30.6%) patients

Voriconazole (n=53 patients)
  Intolerance and / or toxicity
  Clinical failure and / or documented resistance
	N=42 (79.2 %)
	N=28 (52.8%); documented resistance was seen in 14 (26.4%) patients

Posaconazole (N=29 patients)
  Intolerance and / or toxicity
  Clinical failure and / or documented resistance
	N=8 (27.6%)
	N=18 (62.1%); documented resistance was seen in 12 (41.4%) patients

Treatment changed due to funding issues	N= 3 (10.3%)
Baseline renal function** Grade 1 Grade 2 Grade 3a Grade 3b to Grade 5	 N= 48 N= 20 N=3 N= 0

Abbreviations:
TB, Tuberculosis; NTM, Non tuberculous mycobacterial infection; COPD, Chronic obstructive pulmonary disease; ABPA, Allergic bronchopulmonary aspergillosis.
* includes severe pneumonia requiring admission to intensive care (n=3), extrinsic allergic alveolitis (n=1), exposure to silica (n=1) and congenital cystic lung disease (n=1)
** According to KDIGO 2012 categories of chronic kidney disease (KDIGO 2012[B])

Table 2

The clinical response to the first short course of LAmB therapy.

Clinical response observed in the symptom group	Mean number of symptoms that improved	Number (%) of patients that responded
Respiratory*	1.88	39 (60.0%)
Constitutional	1.98	30 (46.1%)
Both respiratory and constitutional	3.17	21 (32.3%)
Either respiratory or constitutional	2.81	27 (41.5%)
No response	1.12	17 (26.1%)

* Nine patients with haemoptysis underwent bronchial artery embolization whilst receiving LAmB therapy.

Table 3

The clinical response to repeated short courses of LAmB therapy.

Clinical response observed in the symptom group*	Mean number of symptoms that improved with each course of treatment	Number (%) of treatment courses where a clinical response was observed
Respiratory	1.68	26 (55.3%)
Constitutional	1.63	24 (51.1%)
Both respiratory and constitutional	3.26	15 (31.9%)
Either respiratory or constitutional	3.0	21 (44.7%)
No response	1.36	11 (23.4%)

*Twenty patients received two or more short-courses of LAmB therapy (2 courses, n=14; 3 courses, n=5; 4 courses, n=1; total number of courses given was 47). This table summarises the clinical response to LAmB therapy for all the courses of treatment they received.

Figure 1

Changes in the St George's Respiratory Questionnaire QOL score after LAmB
treatment in 40 patients
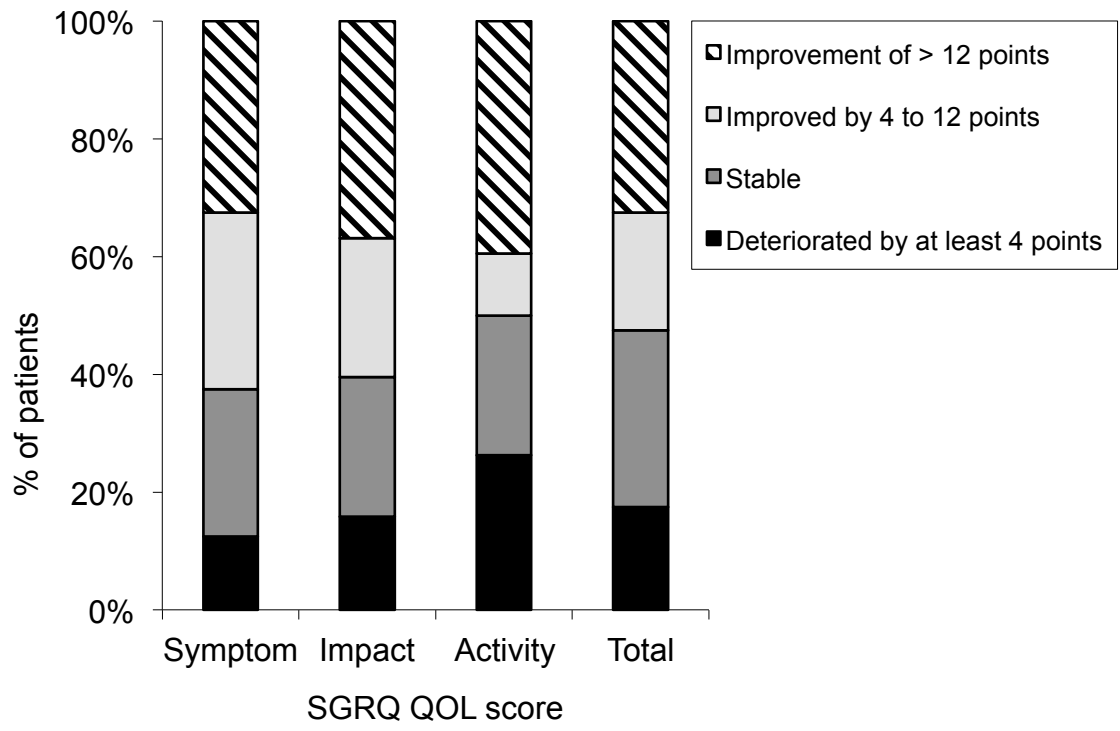
Figure 2
A 5 Kg weight change following repeated courses of LAmB treatment in 13 patients.
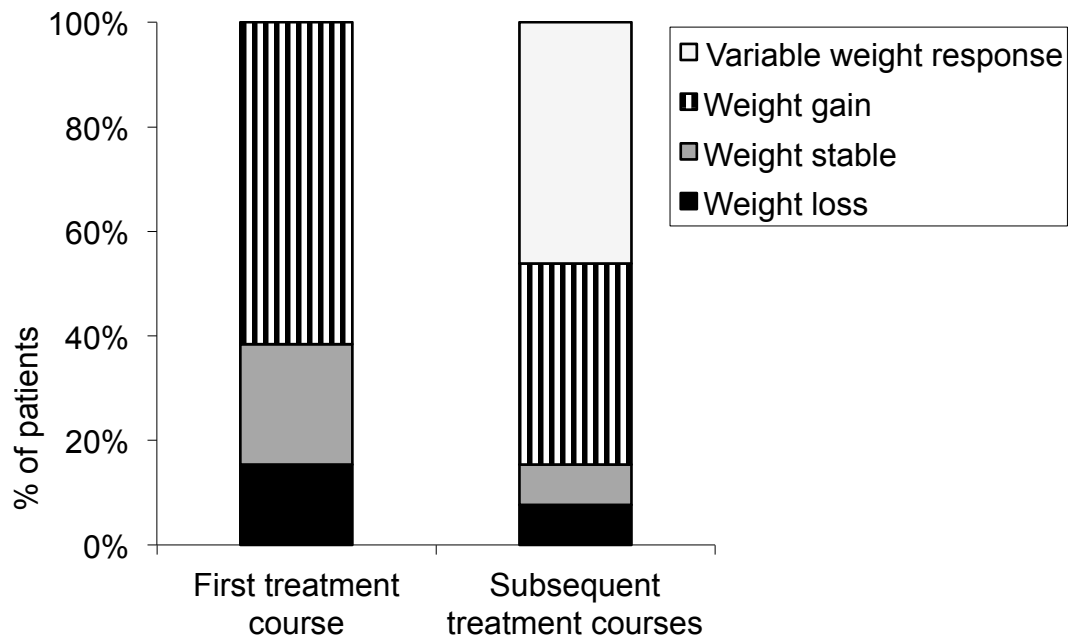
Weight response with IV LAmB treatment

Figure 3
St George's Respiratory Questionnaire QOL changes following repeated courses of
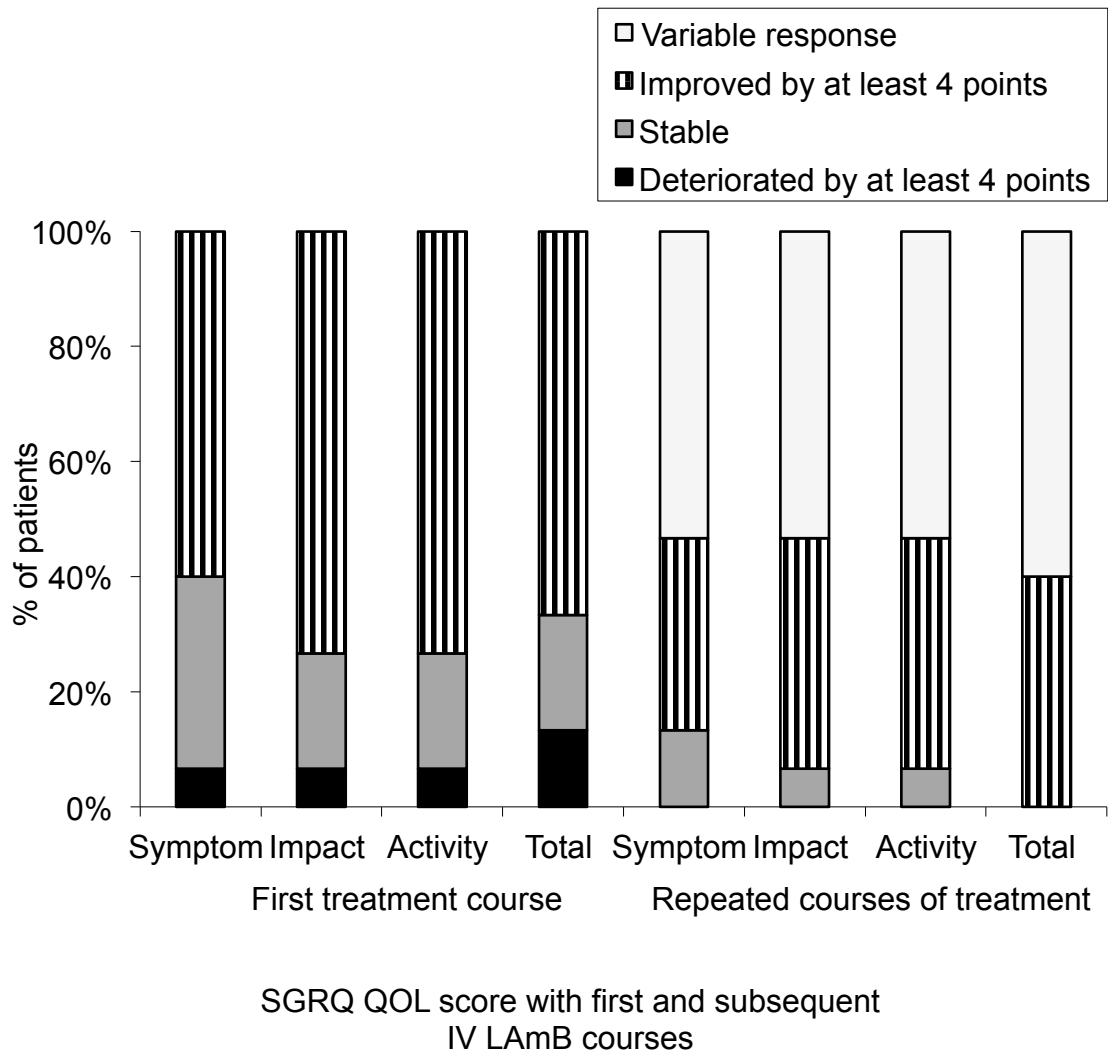LAmB treatment in 13 patients.

SGRQ QOL score with first and subsequent
IV LAmB courses

Figure 4A and 4B
The proportion of patients who developed a risk of an AKI or an AKI during their first course of IV LAmB treatment. Figure 4B refers to those 54 patients whose renal function was assessed beyond day 15**.**
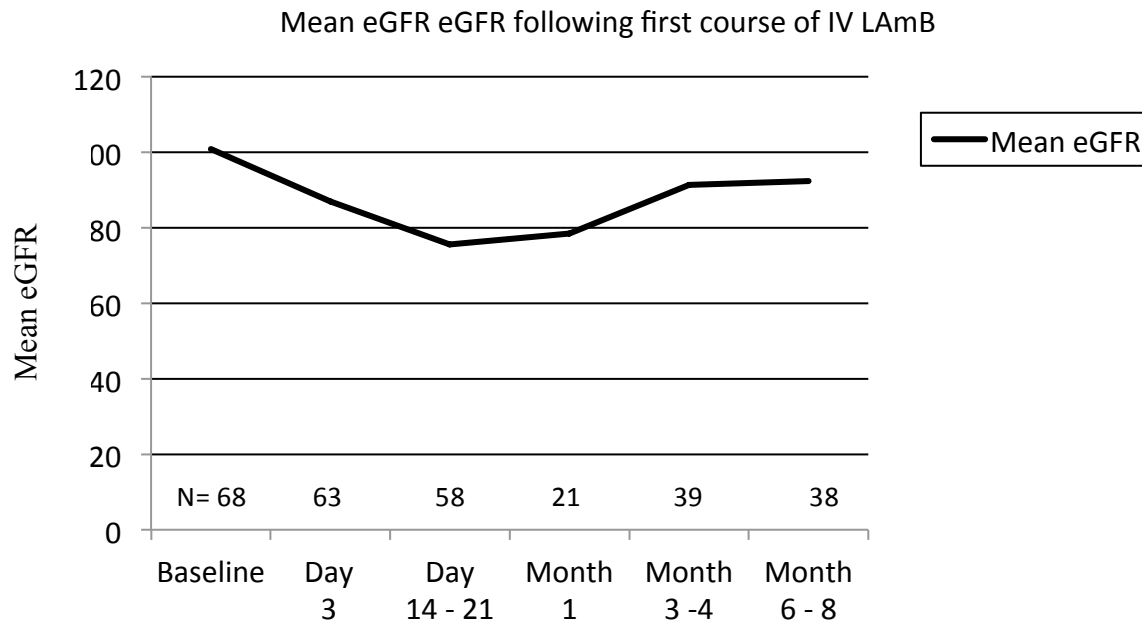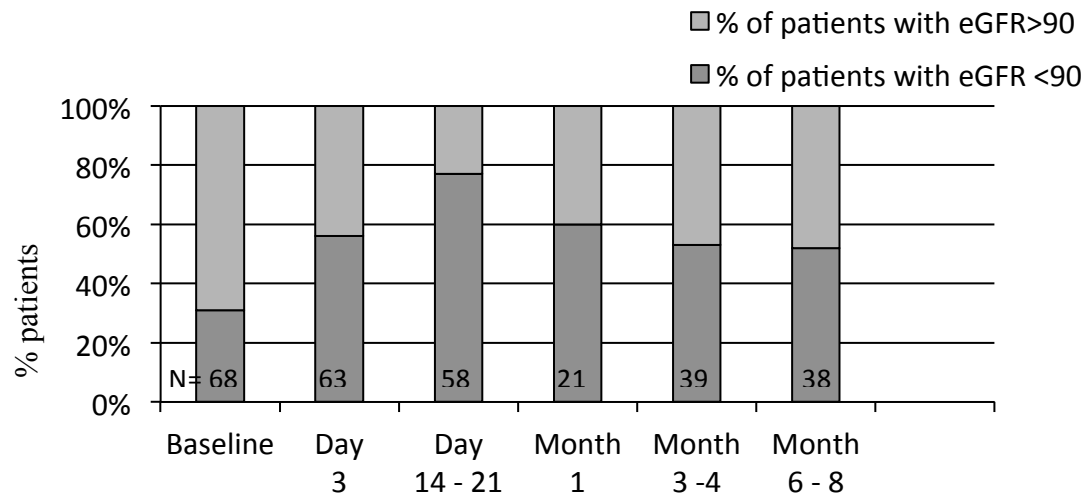
Figure 4A
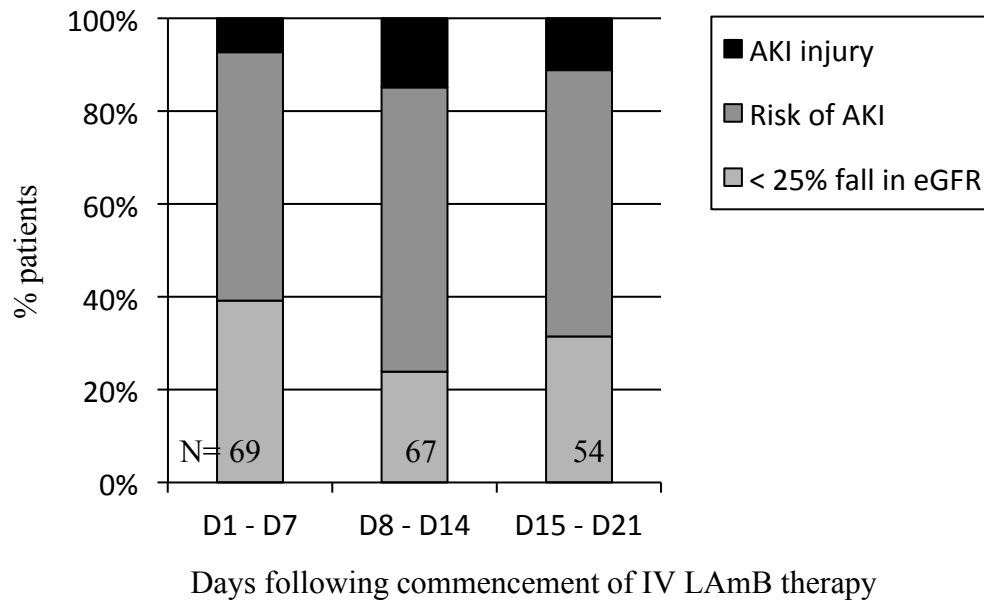
Days following commencement of IV LAmB therapy

Figure 4B

Days following commencement of IV LAmB therapy
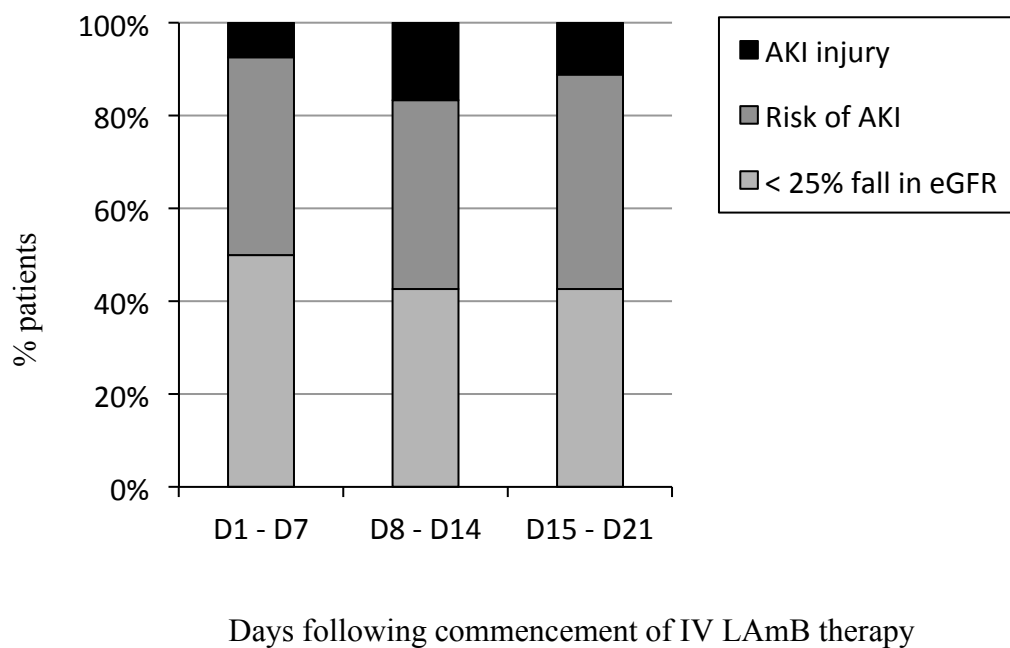
Adjusted geometric mean eGFR observed in patients during and after their first course of LAmB therapy.

Figure 5B
Adjusted percentage of patients with an eGFR less than 90 during and post their first course of LAmB therapy.

This data relates to the 68 patients who received at least 1 dose of LAmB therapy. Renal function post treatment was recorded for up to 8 months following therapy and the data at subsequent time-points was excluded from the analysis if patients had been commencing on a further short-course of LAmB therapy or intermittent long-term treatment.

Adjusted geometric mean eGFR observed in patients during and after their second course of LAmB therapy.
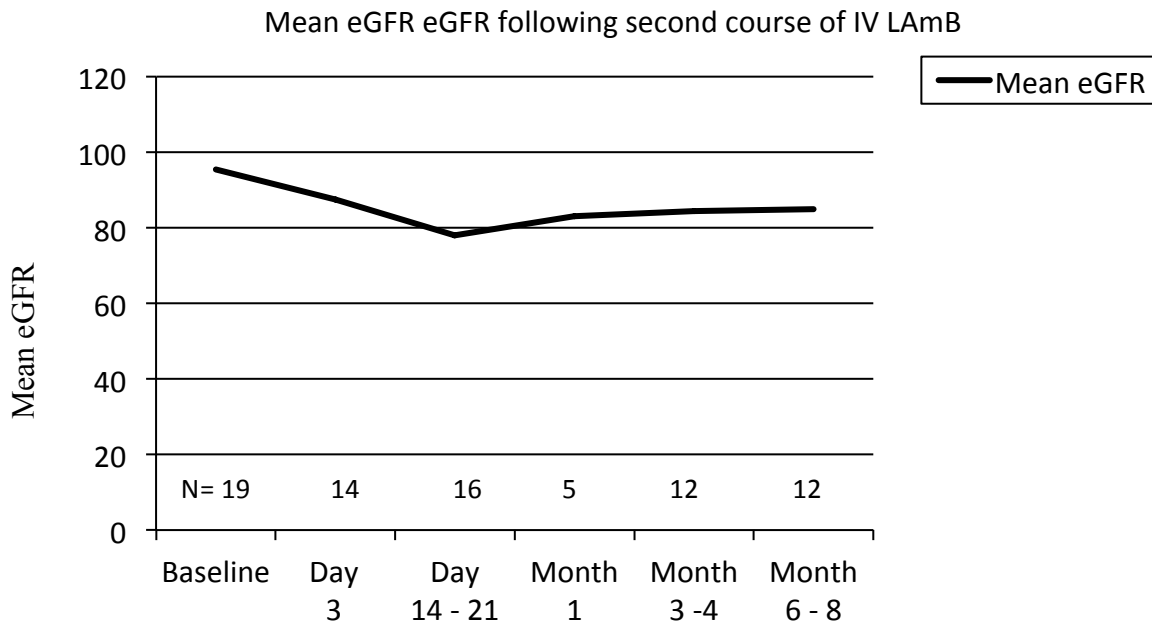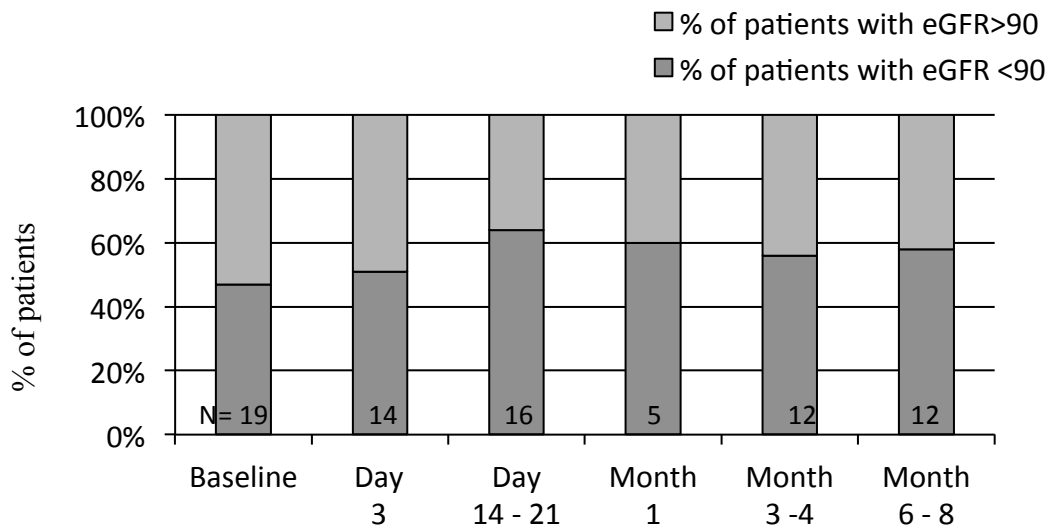

Figure 6B
Adjusted percentage of patients with an eGFR less than 90 during and post their second course of LAmB therapy.

Submission preparation:

Figures – printed in black and white
Print – black and white