Clinical and Radiographic Features of Cryptococcal Neoformans Meningitis-associated Immune Reconstitution Inflammatory Syndrome

Gang Wu1,5 ✉, Xiumei Guo2,5, Yan Wang3 & Zhijian Hu4

Cryptococcal meningitis is the most common intracranial infectious fungal disease. After a period of antifungal treatment, as the number of cells in the cerebrospinal fluid decreases, the biochemical indexes improve and the number of cryptococcus reduces, the patient's condition suddenly worsen. Most of the symptoms are severe headache, raised intracranial pressure, together with impaired clinical nerve function. These presentations are often mistaken for a failure of antifungal treatment. In fact it's an encephalitis syndrome which is unrecognized by most clinicians: Immune reconstitution inflammatory syndrome (IRIS). To increase awareness we retrospectively analyzed clinical data of 100 cases of cryptococcal neoformans meningitis, among which 26 patients develop CM-IRIS. All patients have been divided into three groups: Group 1, patients who were not treated with glucocorticoid and didn't experienced IRIS; Group 2, patients who were not treated with glucocorticoid although developed CM-IRIS; Group 3, patients started treatment with glucocorticoid for two weeks with new onset CM-IRIS. Compared with the group treated with glucocorticoid, treatment without glucocorticoid was subjected to a higher risk of incident IRIS. The difference was statistically significant (P < 0.05).

Imaging findings demonstrated diseased area of the white matter area, and it looked like commonly in the supratentorial region. Moreover, if it appears in the infratentorial region then must be combined with supratentorial region.

Objective. Immune reconstitution inflammatory syndrome (IRIS) describes an abnormal modulation that occurs in the recovery of Immune function in immunocompromised patients. This definition elicits in the process of treating HIV-infected patients and was first proposed by DeSimone J.A. et al. in 20001. Since then, it's been widely cited2. IRIS can not only be appeared in HIV-infected patients4,5, but also be seen in the treatment of non-HIV infected patients with cryptococcal meningitis5,6. We have noticed that cryptococcal meningitis (CM), which is a globally invasive fungal disease with a high morbidity and mortality rate, is often concurrent with IRIS, namely cryptococcal neoformans meningitis-associated immune reconstitution inflammatory syndrome (CM-IRIS). CM-IRIS is generally under-diagnosed, which resulted in delayed treatment. Accurate and timely diagnosis is essential and glucocorticoid is found effective7 to IRIS. To explore the clinical diagnosis and treatment, we conducted a retrospective study of 100 patients diagnosed as cryptococcal neoformans meningitis, finding that a significant number of these patients developed immune reconstitution encephalitis syndrome.
**Materials and Methods**

**Clinical data.** From December 2001 to December 2013 we enrolled 193 patients presented with cryptococcal meningitis and admitted to the Department of Neurology, the First Affiliated Hospital of Fujian Medical University. There were 118 men aged 45.41 ± 14.36, 75 women for ages 41.87 ± 16.09. The cases collected in this study have been approved by the Medical Ethics Committee of the First Affiliated Hospital of Fujian Medical University. And all cases met the diagnosis of cryptococcal meningitis by Azure T. Makadzange, et al.8 The informed consent was obtained from all subjects and their family.

**Inclusion criteria, exclusion criteria, and grouping of cases of cryptococcal meningitis in this study.** Initially 193 patients were enrolled based on the inclusion criteria and exclusion criteria as follows: Inclusion criteria: 1. Hospital stay ≥ 40 days; 2. At least twice (upon admission and discharge) cranial MRI scan; 3. A complete record of therapeutic interventions that serum and cerebrospinal fluid are recorded every fortnight. Exclusion criteria: 1. Cryptococcal meningitis in HIV-Infected patients (4 cases); 2. Length of stay ≤ 40 days (63 cases); 3. Combined with autoimmune diseases, such as systemic lupus erythematosus (SLE), erythodermic psoriasis (18 cases); 4. No complete record of intervention (10 cases); 5. Duplicated data (5 cases). According to all the above-mentioned inclusion and exclusion criteria, 100 patients met these requirements and were included in the final analysis. There were 59 males aged 44.17 ± 12.69 and 41 females aged 42.10 ± 15.88, as shown in Fig. 1.

**Diagnostic criteria for non-HIV IRIS.** The diagnosis of CM-IRIS in this study is in compliance with the diagnostic criteria of non-HIV IRIS proposed by Nina Singh et al. 20079. Of all the included patients in the study, 26 met the diagnostic criteria for HIV-negative CM-IRIS, including 15 men aged 49.93 ± 9.47 years old and 11 women aged 43.91 ± 20.35. 74 patients reported non-IRIS cryptococcal meningitis (CM without IRIS), including 44 males (aged 42.20 ± 13.14) and 30 females (aged 41.43 ± 14.26). All cases were detected by MRI on FLAIR imaging, T2WI imaging and T1WI imaging to check whether abnormal inflammatory signal exists or not. The inflammatory signal obviously decreased or even disappeared after treating with Glucocorticoid (GCs), as shown in Fig. 2 (Fig. 2. A-F). Different inflammatory area divides into 3 types: supratentorial and infratentorial lesions (Fig. 2. G-J) and mixed type. Mixed type means both supratentorial and infratentorial regions were affected, and most of the situations is white matter lesions. Once infratentorial white matter damage developed, it must be combined with supratentorial white matter lesions. Among the 26 cases, 20 cases (76%) are supratentorial lesions and 6 belong to mixed type, no pure infratentorial white matter lesions observed.

**Methods.** **Laboratory data sources.** Data including cerebrospinal fluid biomarkers, biochemical and cytological results was based on the clinical examination data from the clinical laboratory of the First Affiliated Hospital of Fujian Medical University and the Center for Cerebrospinal Fluid Examination of Fujian Province. The imaging data was provided by the Department of Radiology, the First Affiliated Hospital of Fujian Medical University. GE’s SIGNA EXCITE 1.5 T Twin Speed and SIEMENS’s 3.0 T MEGNETOM Verio were adapted as magnetic resonance imaging (MRI) scanner during the study.

**Research design.** We performed a retrospective study. First of all, we compared the baseline data of these subgroups and checked if any difference exists on gender ratio, ages and length of stay: CM-IRIS and CM non-IRIS, CM-IRIS treated with GCs and without GCs. Besides, 100 cases applied not only to the including criteria but also to the excluding criteria were collected to do statistical induction on clinical symptoms, clinical signs and
Cerebrospinal fluid components. Secondly, we divide antifungal drugs into three comparison combinations (A, B and C) to examine their therapeutic effects to CM-IRIS. Then we replicated our analysis according to whether the patient developed IRIS and treated with GCs. Dynamic observation was performed in patients

Figure 2. Supratentorial lesions as showed in picture A, B, C, D; Picture A, B, C are T1W1 imaging, T2W1 and Flair imaging respectively of a patient diagnosed with CM before being treated with GCs, 43th days after being admitted to hospital. T1W1 displayed low signal while T2W2 and Flair imaging displayed high signal which distributed beneath the left frontal cortex, within the white matter of basal ganglia and the right cistern (as indicated by arrows). Picture D, E, F are the same T1W1 imaging, T2W2 and Flair imaging of the patient after being treated with GCs for 21 days. No significant abnormality was seen in T1W1 and T2W2 imaging while the high signal in the left frontal cortex and the white matter of right cistern showed by Flair was obviously decreased or even disappeared in comparison to the previous (relatively compare with the area indicated by arrows in Picture A, B, C). Infratentorial lesions showed in picture G, H, I, J by Flair, T1W1, T1W2 and DWI imaging, the yellow arrow represents inflammatory signal. In this figure they weren't the same person for pictures of supratentorial and infratentorial lesions.
Table 1. The baseline characteristics of patients with CM and developed with IRIS or not, and patients confirmed with CM-IRIS and treated with GCs or not. * Note: For economical reason 4 of the 5 patients in the group of CM-IRIS treated without GCs were discharged from the hospital and transferred to local hospital for further treatment, thus the average hospital stay was short. P1 was the P value for the data of CM-IRIS and the CM non-IRIS group; P2 was the P value for the data of the two groups that CM-IRIS with GCs treatment and without. Both P1 and P2 were above 0.05, hence the data of gender, age and length of hospital stay didn't reach statistical significance.

| Group                  | CM-IRIS (n = 26) | CM non-IRIS (n = 74) | CM-IRIS with GCs (n = 21) | CM-IRIS (without GCs) (n = 5) | P1, P2 |
|------------------------|------------------|----------------------|---------------------------|------------------------------|--------|
| Male to female ratio   | 15:11            | 44:30                | 14:7                      | 2:3                          | 0.875 0.340 |
| Median age             | 47.38 ± 15.00    | 41.89 ± 13.51        | 45.43 ± 14.89             | 55.603 ± 13.90              | 0.086 0.178 |
| Average hospital stay  | 130.77 ± 88.46   | 121.09 ± 70.74       | 146.57 ± 89.70            | 64.00 ± 43.08*              | 0.576 0.060 |

Different combinations of antifungal treatments have been shown to have no effects on not only the treatment of CM but also incident CM-IRIS and CM non-IRIS. In this study we identified the methods for evaluation of effective treatments to people with CM, that is, clinical symptoms and cerebrospinal fluid biomarkers (Supplementary data, Table 1). Three combinations of antifungal treatments in this study are as follows: Group A: Voriconazole/Fluconazole + 5-Fluorocytosine; Group B: Amphotericin B + 5-Fluorocytosine; Group C: Voriconazole/Fluconazole + Amphotericin + 5-Fluorocytosine. We adopted χ² which is a table contains a matrix of rows and columns to conduct the examination: χ² = 11.802, P = 0.299. Statistical results suggested that different combinations of antifungal treatments had been shown to have no effects on treatment to CM; Through adopting χ² (χ² = 0.696, P = 0.706) to do the similar examination: we found the same results in the treatment of different antifungal drugs to CM-IRIS and CM-no IRIS (Supplementary data, Table 2).

To compare three stages of CM-IRIS according to the progression of disease and whether treated with GCs or not. In this study 26 patients confirmed with CM-IRIS were divided into three groups: Group 1: haven't developed IRIS yet and not treated with GCs; Group 2: have developed IRIS and not treated with GCs; Group 3: have developed IRIS and treated with GCs for two weeks. We made comparison on the changes of intracranial pressure of cerebrospinal fluid, glucose, protein, chloride, the leucocytes and lymphocyte percentage. Firstly, we adopted analysis of variance (statistical analysis) to do the analysis, the result indicated that there were significantly different changes of intracranial pressure of cerebrospinal fluid, glucose, protein, chloride, the leucocytes and lymphocyte percentage of the three groups (F values 6.958, 5.344, 0.008, 4.005 and 6.320 respectively, P values 0.002, 0.008, 0.024, 0.003 respectively). On the basis of analysis of variance, we adopted LSD-t test to do data comparison among groups, the results showed intracranial pressure of cerebrospinal fluid of CM-IRIS is obviously higher than CM non-IRIS group (t = 3.168, p < 0.05), also obviously higher than CM-IRIS treated with...
G Cs (t = 3.290, p < 0.05), the difference is significant. With regard to protein of cerebrospinal fluid, the leucocytes and lymphocyte percentage, the protein of CM IRIS group treated without GCs was obviously less than CM non-IRIS group (t = 3.128, p < 0.05), as well as leucocyte count, that was to say Group 1 was higher than Group 2 (t = 2.308, p < 0.05), more than Group 3 (t = 2.348, p < 0.05) too, the difference is of overt significance. As for lymphocyte percentage, compared with the CM-IRIS group, CM non-IRIS (haven't developed IRIS yet) group was higher, that's, Group 2 is higher than Group 1 (t = 2.779, p < 0.05), furthermore Group 3 is higher than Group 1 too (t = 3.056, p < 0.05), the difference was significant. Above-mentioned results can be found in Table 2, Fig. 3.

Table 2. To compare clinical biochemical indicators of the three periods of 26 cases with CM-IRIS. Note: Grouping: Group 1: haven't developed IRIS yet and not treated with GCs; Group 2: have developed IRIS and not treated with GCs; Group 3: have developed IRIS and treated with GCs for two weeks. Statistical methods: Firstly, we adopted analysis of variance (statistical analysis) to do the analysis, the result indicated that there were significantly different changes of intracranial pressure of cerebrospinal fluid, protein, the leucocytes and lymphocyte percentage of the three groups, P values were all less 0.05. Subsequently we made comparison between each group, asterisks (*) and triangles (▲) means statistically significant, of which the P values were all less than 0.05.

To explore the impact whether CM patients treated with or without GCs have on the incidence of CM-IRIS. In this study both CM-IRIS and CM non-IRIS were divided into Group A and Group B according to whether treated with or without GCs. Group A's incidence of IRIS was higher than Group B's (χ² = 6.021, P = 0.014), the difference was significant. As shown in Table 3.

The timing of onset CM-IRIS throughout the whole course of CM. We examined the timing of new onset IRIS of all 21 patients with CM-IRIS. Since the onset of CM, which generally begins with symptoms of headache, fever and other known clinical symptoms, following antifungal treatment, some patients are cured, and a considerable number of patients still fail to treat10. After a period of antifungal treatment, as the number of cells in the cerebrospinal fluid decreases, the biochemical indexes improved and the number of cryptococcus reduces, the patient's condition suddenly worsen. Most of the symptoms are severe headache, raised intracranial pressure, together with impaired clinical nerve function. These presentations are often mistaken for a failure of antifungal treatment. In fact it's an encephalitis syndrome which is unrecognized by most clinicians: Immune reconstitution inflammatory syndrome (IRIS). Available data indicate that up to 20% of patients with complication of cryptococcal meningitis (CM) die from concurrent immune reconstitution encephalitis syndrome11.

In addition to improving the clinical symptoms, we found that GCs has a special effect on this disease in this study. As shown in Table 3, compared with Group 3 which didn't treated with GCs, the intracranial pressure of cerebrospinal fluid of Group 2 that treated with GCs was obviously reduced, as well as clinical symptoms got better. P values were all less than 0.05 therefore the difference was significant.

Data from this study showed that when IRIS occurred, clinical characteristic changed and started with growing headache, increased pressure of cerebrospinal fluid, protein and leucocytes decreased but lymphocyte percentage rose. After using GCs headache was immediately relieved. Two weeks later only to find the pressure of the cerebrospinal fluid obviously went down, no significant changes for other ingredients. No matter treated with GCs or not the serum inflammatory indexes of CM-IRIS didn't reach statistically significance, which was consistent with the views of Chang C. C et al.12. CM can also experience with immune reconstitution inflammatory syndrome (CM-IRIS) in an early stage5. Due to the lack of characteristic manifestations of cerebrospinal fluid, it is more likely to be misdiagnosed.
Therefore, the brain IRIS occurs at this time, and the imaging basis occupies a very important position. The cases collected in this study are not early cases, therefore they are not difficult to identify clinically. The average time of brain IRIS occurred in this study was 47.50 ± 28.44 days, and the predilection time span was 20–60 days. The literature reports that brain IRIS of cryptococcal meningitis often occurs around 6 weeks after antifungal treatment. Premature use of GCS in CM antifungal therapy may affect antifungal therapy. Therefore, based on the average time of brain IRIS occurred in this group and the time of brain IRIS reported in the literature, it may be reasonable to recommend the addition of GCs for about 6 weeks during antifungal therapy for CM.

In addition, according to the imaging results, CM-IRIS's imaging abnormalities occurred in the brain parenchyma on the cerebellum, pure supratentorial lesions were more common, pure infratentorial lesions hadn't been observed. This study shows that once the lesions appeared under the curtain, it must be combined with the supratentorial lesions.

Table 3. To explore the impact if CM patients treated with or without GCs have on the incidence of CM-IRIS. Note: Group A: treated without GCs; Group B: treated with GCs. Adopted χ2 test which contained a matrix of rows and columns to check the formula: χ² = 6.021, P = 0.014.
In summary, through analyzing the clinical symptoms, routine cerebrospinal fluid biomarkers and imaging changes, CM-IRIS of the brain can be correctly identified. Simultaneously, correct antifungal therapy combined with timely GCs treatment can significantly alleviate or reduce the occurrence of brain IRIS.

Received: 29 April 2019; Accepted: 26 May 2020;
Published online: 19 June 2020

References
1. DeSimone, J. A., Pomerantz, R. J. & Babinchak, T. J. Inflammatory reactions in HIV-1–infected persons after initiation of highly active antiretroviral therapy. J. Annals of internal medicine. 133(6), 447–454 (2000).
2. Sun, H. Y. & Singh, N. Immune reconstitution inflammatory syndrome in non-HIV immunocompromised patients. J. Current opinion in infectious diseases 22(4), 394–402 (2009).
3. Eduardo Rodrigues da Cunha Colombo Delio José Mora Mario León Silva-Vergara. Immune reconstitution inflammatory syndrome (IRIS) associated with Cryptococcus neoformans infection in AIDS patients. J. Mycoses. 54: 178-182(2010).
4. Shelburne, S. A. III et al. The Role of Immune Reconstitution Inflammatory Syndrome in AIDS-Related Cryptococcus neoformans Disease in the Era of Highly. Active Antiretroviral Therapy J. HIV/AIDS. 40, 1049–1052 (2005).
5. Ecevit, I. Z. et al. The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. Clin Infect Dis 42, 1443–1447 (2006).
6. Panackal, A. A. et al. Paradoxical Immune Responses in Non-HIV Cryptococcal Meningitis. J. PLOS Pathogens. 11(3), 1004884 (2015).
7. Perfect, J. R. et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. J. Clinical Infectious Diseases. 50(3), 291–322 (2010).
8. Azure T. M. G McHugh, New Approaches to the Diagnosis and Treatment of Cryptococcal Meningitis, Seminars in Neurology. 34 (1):47-60(2014).
9. Singh, N. & Perfect, J. R. Immune reconstitution syndrome associated with opportunistic mycoses.J. The Lancet infectious diseases. 7(6), 395–401 (2007).
10. Day, J. N. et al. Combination antifungal therapy for cryptococcal meningitis. J. New England Journal of Medicine. 368(14), 1291–1302 (2013).
11. Musubire, A. K. et al. Challenges in diagnosis and management of Cryptococcal immune reconstitution inflammatory syndrome (IRIS) in resource limited settings. J. African health sciences. 12(2), 226–230 (2012).
12. Chang, C. C. et al. Clinical and mycological predictors of cryptococcus-associated immune reconstitution inflammatory syndrome. J. AIDS. 27(13), 2089–2099 (2013).
13. Rushing, E. J. et al. Immune reconstitution inflammatory syndrome of the brain: case illustrations of a challenging entity.J. Journal of Neuropathology & Experimental Neurology. 67(8), 819–827 (2008).
14. Jenny-Avital, E. R. & Abadi, M. Immune reconstitution cryptococcosis after initiation of successful highly active antiretroviral therapy. Clin Infect Dis. 35, e128–e133 (2002).
15. Perfect, J. R., Dismukes, W. E. & Dromer, F. Clinical Practice Guidelines for the Management of Cryptococcus neoformans Disease in the Era of Highly. Active Antiretroviral Therapy J. HIV/AIDS. 40, 1049–1052 (2005).
16. Phillips, P. et al. Dexamethasone in Cryptococcus gattii central nervous system infection. J. Clinical infectious diseases. 49(4), 591–595 (2009).

Author contributions
G.W. proposed the study design and wrote the main manuscript text. X.G. contributed to the design of the comparison group, performed the main experimental results. Y.W. was the interpreter for the study. Z.H. contributed to statistical analysis for this study. All authors discussed the results and substantially contributed to the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-67031-4.

Correspondence and requests for materials should be addressed to G.W.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020