Secondary haemophagocytic lymphohistiocytosis: Experience from the Uppsala University Hospital

Torbjörn Karlsson

To cite this article: Torbjörn Karlsson (2015) Secondary haemophagocytic lymphohistiocytosis: Experience from the Uppsala University Hospital, Upsala Journal of Medical Sciences, 120:4, 257-262, DOI: 10.3109/03009734.2015.1064500

To link to this article: https://doi.org/10.3109/03009734.2015.1064500

© Informa Healthcare

Published online: 25 Jul 2015.

Submit your article to this journal

Article views: 875

View related articles

View Crossmark data

Citing articles: 7 View citing articles
Secondary haemophagocytic lymphohistiocytosis: Experience from the Uppsala University Hospital

TORBJÖRN KARLSSON

Department of Haematology, Uppsala University Hospital, Uppsala, Sweden

Abstract

Background. Haemophagocytic lymphohistiocytosis (HLH) is a rare clinical syndrome characterized by fever, hepatosplenomegaly, cytopenia, and progressive multiple-organ failure. HLH in adults is often secondary to autoimmune diseases, cancer, or infections in contrast to familial HLH. Treatment of secondary HLH is directed against the triggering disease in addition to immunosuppressive therapy, the latter commonly according to the HLH-2004 protocol.

Methods. We conducted a retrospective study to identify triggering diseases, disease-specific and immunosuppressive therapy administered, and prognosis in adult patients with secondary HLH. Patient data were collected from October 2010 to January 2015.

Results. Ten adult patients with secondary HLH were identified. Seven were men, and the median age at diagnosis was 62 years. Five cases were triggered by malignant disease and five by infection. The median patient fulfilled five of the eight HLH-2004 diagnostic criteria. All patients fulfilled the criteria fever, cytopenia, and ferritin >500 μg/L. Median time from hospital admission to HLH diagnosis was 20 days. Four patients received immunosuppressive therapy according to the HLH-2004 protocol. The prognosis was dismal, especially for the patients with malignancy-associated HLH, of whom all died.

Conclusion. HLH should be suspected in patients who present with fever, cytopenia, and ferritin >500 μg/L. Secondary HLH has a dismal prognosis. None of the patients with HLH triggered by malignancy survived. Achieving remission of the triggering disease seems to be important for a favourable outcome as, in all surviving patients, the haemophagocytic syndrome resolved after remission of the underlying infection.

Key words: Haemophagocytic lymphohistiocytosis, HLH-2004 protocol

Introduction

Haemophagocytic lymphohistiocytosis (HLH) is a rare clinical syndrome characterized by fever, hepatosplenomegaly, cytopenia, and progressive multiple-organ failure (1). Secondary HLH is often triggered by autoimmune diseases, malignancy, or infection (1). The pathogenetic mechanisms behind HLH are not completely understood but involve defective granule-mediated cytotoxicity and uncontrolled T-cell activation, leading to an exaggerated inflammatory response (2), the consequence of which is tissue damage and progressive multiple-organ failure. The spleen, liver, and lungs are the most frequently affected organs (1), but HLH can involve virtually all tissues and organs in the body. In contrast to familial HLH, which often (but not always) presents in paediatric age (3), secondary HLH predominantly occurs in adults. Secondary HLH is arbitrarily divided into three groups depending on triggers and associated diseases. Autoimmune disease-associated HLH is denoted A-HLH, whereas HLH triggered by malignancy and infection is denoted M-HLH and I-HLH, respectively. M-HLH is often associated with haematologic malignancies, and the annual incidence of M-HLH in Sweden is less than 0.4 in 100,000 (4). I-HLH is predominantly triggered by Epstein–Barr virus (EBV) or cytomegalovirus (CMV) (1). Bacterial
infections are reported less frequently than viruses as HLH triggers, and mycobacteria dominate among bacteria associated with I-HLH (5).

The HLH diagnostic guidelines proposed by Henter et al. in 1991 (6) were updated in 2004 (7). A patient is diagnosed with HLH if there is a molecular diagnosis consistent with HLH; that is, pathological mutations of, for example, PRF1, UNC13D, or STX11, or if at least five of the following eight criteria are fulfilled: fever, splenomegaly, cytopenia, hypertriglyceridaemia/hypofibrinogenaemia, haemophagocytosis in biopsy, low/absent natural killer (NK) cell activity, hyperferritinaemia, and elevated soluble CD25 receptor (sCD25R). These diagnostic guidelines are commonly used in both familial and secondary HLH.

With a mortality rate of approximately 40% (1), the HLH patient is often critically ill with progressive multiple-organ failure, requiring the resources of an intensive care unit. Thus, it is essential to support the vital functions of the patient while identifying and subsequently eliminating possible HLH triggers, for example, chemotherapy for a lymphoma or antibiotics for an infection. Simultaneously, the exaggerated inflammatory response must be treated with immunosuppressants. Although chemotherapy (8), monotherapy with glucocorticoids (9), and intravenous immunoglobulins (10) have been used, a combination of glucocorticoids and chemotherapy is currently recommended as first-line HLH treatment (7). The HLH-2004 protocol, which was developed for use in the paediatric HLH setting, consists of a combination of dexamethasone, cyclosporine A, and etoposide (7).

No trials using the HLH-2004 protocol have been performed in adults, but this protocol is widely used as first-line treatment of secondary HLH.

Patients and methods

From October 2010 to January 2015 we identified nine adult patients who were diagnosed with and treated for secondary HLH at the Department of Haematology, Uppsala University Hospital. One patient who was diagnosed with HLH at another hospital, but referred to Uppsala University Hospital for treatment, was also included in this study. The HLH diagnosis was based on the HLH Study Group of the Histiocyte Society 2004 criteria (7). According to the HLH-2004 guidelines, five of the following eight criteria must be fulfilled for the HLH diagnosis: 1) fever (temperature >38.5°C); 2) splenomegaly; 3) cytopenias affecting ≥2 of three lineages (Hb <90 g/L, absolute neutrophil count [ANC] <1×10⁹/L, platelet count <100×10⁹/L); 4) plasma triglycerides ≥3 mmol/L and/or fibrinogen ≤1.5 g/L; 5) haemophagocytosis in biopsy from bone marrow, spleen, or lymph node; 6) plasma ferritin ≥500 μg/L; 7) low or absent NK-cell activity; and 8) sCD25R ≥2400 U/mL. The case records of the patients were reviewed, and clinical, histopathological, and laboratory data at the time of the HLH diagnosis were identified, as were the diseases triggering the haemophagocytic syndrome for each patient. Time from admission to hospital to HLH diagnosis, disease-specific treatment, and prognosis were also recorded. We also studied whether or not the patients had been treated according to the HLH-2004 protocol. This study was approved by the Regulatory Ethics Committee of Uppsala and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained for all patients. Statistical analyses were performed using the SigmaPlot 11 software package (Systat Software, San Jose, CA, USA). Quantitative variables were expressed as median (range).

Results

Ten patients were treated for secondary HLH at the Department of Haematology, Uppsala University Hospital between October 2010 and January 2015. Seven of them were men, and the median age was 62 (range 30–75) years. Five of the patients were diagnosed with a triggering malignant disease (four cases of lymphoma and one case of cancer of unknown primary [CUP]). CMV/EBV and mycobacterial infection were diagnosed in three and two patients, respectively (Table I). Median time from admission to hospital to HLH diagnosis was 20 (range 5–60) days, and the median patient fulfilled five (range 5–7) of the eight HLH-2004 diagnostic criteria. At the time of admission to hospital, all patients suffered from fever and cytopenia (Table I) and exhibited biochemical signs of inflammation as determined by elevated C-reactive protein (CRP) and erythrocyte sedimentation rate. All 10 patients received empiric broad-spectrum antibiotic therapy upon admission to hospital, and four of them also received antifungal therapy. Median CRP was 129 (range 14–205) mg/L. Ferritin was above 500 μg/L in all patients, and sCD25R exceeded 2400 U/mL in the five patients tested. Splenomegaly and/or haemophagocytosis in bone marrow or lymph node biopsies were present in all but two patients. Triglycerides were elevated in four patients, but there was hypofibrinogenaemia in only one of nine patients tested. Since tests for NK-cell activity are not available at the Uppsala University Hospital, this test was performed in two patients only. NK-cell activity was low and analysis inconclusive in patients number 6 and 10, respectively (Table I). Analysis of peripheral
blood values revealed that all 10 patients were anaemic, with a median haemoglobin concentration of 98 (range 89–122) g/L. The median ANC was 0.7 (range 0.1–45) \( \times 10^9 \)/L. None of the patients had a platelet count above 100 \( \times 10^9 \)/L, and the median platelet count was 27 (range 9–69) \( \times 10^9 \)/L (Table II).

Patients number 1 and 2, diagnosed with diffuse large B-cell lymphoma and CUP, respectively, did not receive any disease-specific chemotherapy or immunosuppression according to the HLH-2004 protocol before they died of HLH-associated multiple-organ failure (Table III). Patient number 3, who was diagnosed with Hodgkin lymphoma, received chemotherapy but no immunosuppression and died of multiple-organ failure. Patients number 4 and 5 were diagnosed with aggressive T-cell non-Hodgkin lymphoma and received CHOP-based chemotherapy and immunosuppression according to HLH-2004. They died during chemotherapy from staphylococcal septicaemia and intracerebral haemorrhage, respectively, without having achieved lymphoma or HLH remission (Table III). Patient number 6 fell ill with a primary EBV infection and CMV reactivation. He received antiviral treatment with rituximab and fosfomycin and immunosuppression according to HLH-2004 but died after 73 days from multiple-organ failure and an invasive Candida infection (Table III). Patient number 7 has been described elsewhere (11). Briefly, this woman was admitted to the hospital with an EBV reactivation with transient EBV viraemia and HLH. Patient number 8 is a man with acute lymphoblastic leukaemia of T-cell type in complete remission who fell ill with HLH secondary to CMV reactivation. Patients number 7 and 8 were successfully treated with dexamethasone and ganciclovir only, respectively. They are alive and without any signs of active HLH after 49 and 4 months of observation, respectively (Table III). Patients number 9 and 10 were diagnosed with a triggering Mycobacterium avium and Mycobacterium tuberculosis infection, respectively. Patient number 9 was treated with dexamethasone and anti-tuberculous drugs only. When he fell ill with HLH, the chronic lymphatic leukaemia he was diagnosed with was in remission, but he suffered from chronic secondary hypogammaglobulinaemia.

| Patient | Sex (M/F) | Age (years) | Disease | Fever | Splenomegaly | Cytopenias | TG/fibrinogen | HP in biopsy | sCD25R | Ferritin | NK-cell activity | HLH criteria |
|---------|-----------|-------------|---------|-------|-------------|-----------|--------------|-------------|--------|----------|--------------|-------------|
| 1       | M         | 75          | NHL     | +     | +           | +         | –/–          | +/ND       | +      | +/ND     | ND Low       | 5/8         |
| 2       | M         | 69          | CUP     | +     | +           | +         | –/–          | +/ND       | +      | +/ND     | ND Low       | 6/8         |
| 3       | M         | 63          | HL      | +     | +           | +         | +/–          | +/–        | +      | +/ND     | ND Low       | 5/8         |
| 4       | F         | 39          | NHL     | +     | +           | +         | +/–          | +/–        | +      | +/ND     | ND Low       | 7/8         |
| 5       | M         | 59          | EBV/CMV | +     | +           | +         | +/–          | +/–        | +      | +/ND     | ND Low       | 5/8         |
| 6       | M         | 34          | EBV     | +     | +           | +         | +/–          | +/–        | +      | +/ND     | ND Low       | 5/8         |
| 7       | M         | 66          | CMV     | +     | +           | +         | +/–          | +/–        | –      | +/ND     | ND Low       | 5/8         |
| 8       | F         | 30          | MB      | +     | +           | +         | +/–          | +/–        | –      | +/ND     | ND Low       | 5/8         |
| 9       | M         | 61          | MB      | +     | +           | +         | +/–          | +/–        | –      | +/ND     | ND Low       | 5/8         |
| 10      | M         | 30          | MB      | +     | +           | +         | +/–          | +/–        | –      | +/ND     | ND Low       | 5/8         |

+ = Present or pathologically increased/decreased; – = Absent or normal; AI = Analysis inconclusive; CMV = Cytomegalovirus infection; CUP = Cancer of unknown primary; EBV = Epstein-barr virus infection; F = Female; HL = Hodgkin lymphoma; HLH, Haemophagocytic lymphohistiocytosis; HP = Haemophagocytosis; M = Male; MB = Mycobacteria infection; ND = Not determined; NHL = Non-hodgkin lymphoma; sCD25R = Soluble CD25 receptor; TG = Triglycerides.
After 18 months of observation he exhibits no signs of mycobacterial infection or active HLH (Table III). Patient number 10 was diagnosed with acute myelogenous leukaemia. When he fell ill with HLH, the leukaemia was in complete remission. Initially, he received intravenous immunoglobulin treatment without any effect. He died of acute liver failure a few days after initiation of immunosuppression according to HLH-2004, and 18 days after that the HLH diagnosis was made (Table III).

Three of the patients exhibited neurological symptoms suggestive of cerebral HLH. Patients number 3 and 6 fell into stupor, and patient number 10 suffered from multiple epileptic seizures and a transient paraparesis. Brain magnetic resonance imaging (MRI) showed multiple unspecific focal lesions in the cerebrum and a moderate cerebral oedema in patients 6 and 10, respectively. Analysis of cerebrospinal fluid from patient number 6 revealed pleocytosis and an increased protein level, which strengthens the suspicion of cerebral HLH in this patient. This patient received systemic immunosuppression according to HLH-2004, but no intrathecal therapy.

All five patients with M-HLH died, as did two of the patients with I-HLH. Immunosuppression according to the HLH-2004 protocol did not induce remission of the haemophagocytosis in any of the four patients treated, and they all died (Table III). The median time from HLH diagnosis to death was 22 (range 0–108) days. The three patients who are alive all suffered from I-HLH, and remission of the triggering infection was associated with remission of the haemophagocytic syndrome.

### Discussion

The differential diagnosis between a severe infection and HLH can be difficult, especially since an infection can precede and trigger HLH. It is noteworthy that all 10 patients reported here presented with fever, cytopenia, and elevated CRP, and that all of them were initially treated with empiric broad-spectrum antibiotics and four of them with antifungal therapy. The median time-span of 20 days from admission to hospital to establishment of the HLH diagnosis can probably, at least to some extent, be explained by the fact that the patients presented with symptoms and biochemical signs typical for infection and were initially treated accordingly. Among the five M-HLH patients, bacteria were present in cultures from one patient only. This patient died of septicaemia, whereas the deaths of the other four M-HLH patients were not related to infection. I-HLH is not seen in immunodeficient patients only (7), and two of our five I-HLH patients did not suffer from any obvious pre-existing immunodeficiency. Altogether, an infection was proven in 6 of the 10 patients reported here, but infection was the definitive cause of death in 1 patient only. In two recent reports, HLH was triggered by mycobacteria in less than 10% of the cases (12,13). This is in contrast to what we observe with 20% of the cases triggered by mycobacteria. This discrepancy is probably explained by the fact that our analysis is based on a smaller patient population.

Cerebral HLH is common, and symptoms range from neck stiffness to coma (14,15). Three of the patients reported here exhibited symptoms suggestive of cerebral HLH. In cerebral HLH the most common laboratory findings are pleocytosis and an increased protein concentration in cerebrospinal fluid (14,15). These findings were observed in the one of our patients who was subjected to a diagnostic lumbar puncture. In one single-centre report (15), neuroimaging of patients with cerebral HLH showed no consistent pattern of lesions, but white-matter abnormalities were common. Brain MRI of patient number 10 revealed a cerebral oedema, a finding that is common in post-mortem examination of patients with cerebral HLH (14). In the paediatric setting, systemic therapy according to the HLH protocol often

### Table III. Clinical data and outcome for the 10 individual HLH patients.

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---------|---|---|---|---|---|---|---|---|---|----|
| Time to diagnosis* | 23 | 20 | 10 | 19 | 17 | 17 | 16 | 29 | 60 | 52 |
| HLH-2004* | – | – | CTX | – | CTX | CTX | AVT | Dx | AVT | ATD, Dx |
| Therapy | – | – | CTX | – | CTX | CTX | AVT | Dx | AVT | ATD, Dx |
| Outcome | Dead at d0 | Dead d+10 | Dead d+22 | Dead d+108 | Dead d+36 | Dead d+73 | Alive | Alive | Alive | Dead d+18 |

*Time to diagnosis defined as time (days) from admission to hospital to HLH diagnosis.

**HLH-2004 defined as treatment according to the HLH-2004 protocol.

*Outcome defined as number of days from HLH diagnosis.

+ = Yes; – = No; ATD = Anti-tuberculous drugs; AVT = Antiviral therapy; CTX = Chemotherapy; Dx = Dexamethasone; IVIG = Intravenous immunoglobulins; HLH = Haemophagocytic lymphohistiocytosis.
reduces haemophagocytosis in the central nervous system, and it has not been shown that addition of intrathecal therapy is beneficial (7). Two of our patients with suspected cerebral HLH received HLH-2004 treatment, but we did not observe any convincing regression of their neurological symptoms.

In the HLH-2004 guidelines, three novel diagnostic criteria, namely hyperferritinaemia, elevated sCD25R, and low/absent NK-cell activity, were added to the five criteria in HLH-94 (7). Although a high ferritin level alone is not diagnostic for HLH (16), ferritin >500 µg/L has a high sensitivity for this diagnosis (17). It has previously been reported that patients with M-HLH have very high ferritin levels (4). The level of sCD25R was above the diagnostic cut-off \( \geq 2400 \) U/mL in all patients tested. Diagnostic sensitivity of sCD25R has been reported to be 0.93 in a paediatric population (18), and the levels, which reflect T-cell activity, are rarely very high in conditions other than haematologic malignancies and HLH (19). Haemophagocytosis present in biopsies from bone marrow or lymph nodes must be interpreted with caution, since this phenomenon is observed in a majority of critically ill patients without any other clinical or biochemical evidence of HLH (20). In a single-centre study of patients with acute leukaemia, the prevalence of haemophagocytosis was approximately 9% (21). The authors of that study did not use the Histiocyte Society diagnostic guidelines for HLH, though. In the clinical context, we believe that the haemophagocytosis we observed in biopsies from our patients represents a clinically relevant and pathologic haemophagocytosis and not a physiological one that can be observed in infections and autoimmune diseases (1).

Although the efficacy of the HLH-94 and 2004 protocols has never been tested in adults with secondary HLH in any clinical trial, they are commonly used in this setting. Machaczka and co-workers retrospectively evaluated the efficacy of the HLH-94 protocol in adults with M-HLH (4). Three of six treated patients responded, but only one of them achieved a sustained remission. Treatment according to the HLH-94 protocol has also been used as a bridge to allogeneic stem cell transplantation in M-HLH (22).

In our study population, four patients were treated according to the HLH-2004 protocol. In none of them was remission of the haemophagocytic syndrome achieved, and they all died. It is interesting to note that in all of these four patients lack of response to immunosuppression was associated with lack of remission of the triggering disease. It is plausible that remission of the triggering disease increases the efficacy of immunosuppression directed against the haemophagocytic syndrome. Our results with fever, cytopenias, and hyperferritinaemia observed as the most commonly presenting HLH findings are in agreement with what was observed in a large recently published single-centre HLH study (13), as is the dismal prognosis, especially for patients with M-HLH. Although the number of HLH patients reported here is very limited, it seems that achievement of remission of the triggering disease is important for a favourable outcome of secondary HLH.

Funding:

Declaration of interest: The author reports no conflicts of interest.

References

1. Ramos-Casals M, Brito-Zeron P, Lopez-Guillermo A, Khamashita M, Bosch X. Adult haemophagocytic syndrome. Lancet. 2014;383:1503–16.

2. Canna S, Behrens E. Not all hemophagocytes are created equally: appreciating the heterogeneity of hemophagocytic syndromes. Curr Opin Rheumatol. 2012;24:113–18.

3. Sieni B, Cetica V, Piccin A, Gherlinzoni F, Carlo Sasso F, Rabusin M, et al. Familial hemophagocytic lymphohistiocytosis may present during adulthood: clinical and genetic features of a small series. PLoS One. 2012;7:e44649.

4. Machaczka M, Vaktiás J, Klímkowska M, Hägglund H. Malignancy-associated hemophagocytic lymphohistiocytosis in adults: a retrospective population-based analysis from a single center. Leuk Lymphoma. 2011;52:613–19.

5. Brastianos P, Swanson J, Torbenson M, Sperati J, Karakousis P. Tuberculosis-associated haemophagocytic syndrome. Lancet Infect Dis. 2006;6:447–54.

6. Henter J-I, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL study group of the histiocyte society. Semin Oncol. 1991;18:29–33.

7. Henter J-I, Horne A, Arico M, Egeler M, Filipovic A, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;4:124–31.

8. Shin H-J, Chung J, Lee J-J, Sohn S, Choi Y, Kim Y-Y, et al. Treatment outcomes with CHOP chemotherapy in adult patients with hemophagocytic lymphohistiocytosis. J Korean Med Sci. 2008;23:439–44.

9. Pukaya S, Yasuda S, Hashimoto T, Oku K, Kataoka H, Horita T, et al. Clinical features of haemophagocytic syndrome in patients with systemic autoimmune diseases: analysis of 30 cases. Rheumatology. 2008;47:1686–91.

10. Emmenegger U, Frey U, Reimers A, Fux C, Semela D, Cottagnoud P, et al. Hyperferritinemia as indicator for intravenous immunoglobulin treatment in reactive macrophage activation syndrome. Am J Hematol. 2001;68:4–10.

11. Antonodimitrakis P, Wassberg C, Gerovasileiou S, Back J, Hällgren R, Olsen B. Fulminant hemophagocytic lymphohistiocytosis secondary to a reactivated EBV infection: a case report. Ups J Med Sci. 2013;118:42–5.

12. Riviere S, Galicier L, Coppo P, Marzac C, Aumont C, Lambert O, et al. Reactive hemophagocytic syndrome in
adults: a retrospective analysis of 162 patients. Am J Med. 2014;127:1118–25.
13. Otrock Z, Eby C. Clinical characteristics, prognostic factors, and outcomes of adult patients with hemophagocytic lymphohistiocytosis. Am J Hematol. 2015;90:220–4.
14. Henter J-I, Nennesmo I. Neuropathologic findings and neurologic symptoms in twenty-three children with hemophagocytic lymphohistiocytosis. J Pediatrics. 1997;130:358–65.
15. Haddad E, Sulis M-L, Jabado N, Blanche S, Fischer A, Tardieu M. Frequency and severity of central nervous system lesions in hemophagocytic lymphohistiocytosis. Blood. 1997;89:794–800.
16. Schram A, Campigotta F, Mullally A, Fogerty A, Massarotti E, Neuberg D, et al. Marked hyperferritinemia does not predict for HLH in the adult population. Blood. 2015;125:1548–52.
17. Esumi N, Ishimura S, Todo S, Imashuku S. Hyperferritinemia in malignant histiocytosis, virus-associated hemophagocytic syndrome and familial erythrophagocytic lymphohistiocytosis. A survey of pediatric cases. Acta Paediatr Scand. 1989;78:268–70.
18. Komp D, McNamara J, Buckley P. Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. Blood. 1989;73:2128–32.
19. Kleyberg R, Schiller G. Secondary hemophagocytic lymphohistiocytosis in adults: an update on diagnosis and therapy. Clin Adv Hematol Oncol. 2012;10:726–32.
20. Strauss R, Neureiter D, Westenburger B, Wehler M, Kirchner T, Hahn E. Multifactorial risk analysis of bone marrow histiocytic hyperplasia with hemophagocytosis in critically ill medical patients—a postmortem clinicopathologic analysis. Crit Care Med. 2004;32:1316–21.
21. Delavigne K, Berard E, Bertoli S, Corre J, Duchayne E, Demur C, et al. Hemophagocytic syndrome in patients with acute myeloid leukemia undergoing intensive chemotherapy. Haematologica. 2014;99:474–80.
22. Machaczka M, Nahi H, Karbach H, Klimekowska M, Hägglund H. Successful treatment of recurrent malignancy-associated hemophagocytic lymphohistiocytosis with a modified HLH-94 immunochemotherapy and allogeneic stem cell transplantation. Med Oncol. 2012;29:1231–6.