Circulating and Ex Vivo Production of Pyrogenic Cytokines and Interleukin-1 Receptor Antagonist in 123 Patients with Fever of Unknown Origin

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Circulating and ex vivo production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and IL-1 receptor antagonist (ra) and the diagnostic utility of these cytokines were studied in 123 patients with fever of unknown origin (FUO). Diagnoses were infections, 28; neoplasms, 14; noninfectious inflammatory diseases (NIID), 32; miscellaneous diseases, 10; and none made, 39. IL-1β, IL-6, and IL-1ra concentrations were higher in patients with infections, neoplasms, and NIID than in healthy controls. Patients with infections had higher concentrations of TNF-α than controls. The ex vivo production of IL-1β and IL-1ra in all patients with FUO did not differ from that in controls; however, production of TNF-α was lower in patients with neoplasms and NIID, and IL-6 production was lower in patients with neoplasms. Thirty-five patients with fever did not have elevated cytokines. Although some significant differences were found among the diagnostic subgroups, there was wide variation. Thus, measurement of these cytokines does not aid in the diagnosis of FUO.

Fever of unknown origin (FUO; >38.3°C for >3 weeks and no diagnosis after 1 week of in-hospital evaluation [1]) is a challenging clinical problem. The bona fide endogenous pyrogens are the proinflammatory cytokines interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and interferon-α [2]. These are involved in various immunologic and inflammatory processes and act directly on the temperature regulation center in the hypothalamus [2]. If the elevated temperature in patients with FUO represents genuine fever, the set point at the temperature center in the preoptic area of the hypothalamus is elevated, which, according to the prevailing concept, is mediated by endogenous pyrogens entering the hypothalamus via the bloodstream. Thus, one or more endogenous pyrogens should be present in the circulation in these patients, and measurement of these cytokines could lead to insight in the pathogenesis of fever. In a prospective cohort of patients with FUO, we examined this pathophysiologic concept by measuring IL-1β, TNF-α, and IL-6 in the circulation. We also measured the concentrations of circulating antiinflammatory cytokine IL-1 receptor antagonist (ra), which is thought to be under the same control as IL-1β [3]. In addition, we addressed the question of whether determination of these circulating cytokines or cytokine production capacity of blood cells is useful in the diagnostic process of FUO.

Materials and Methods

Patients. All 167 patients fulfilling the criteria for FUO were studied prospectively from January 1992 until January 1994 in all eight Dutch university hospitals. Blood samples for cytokine measurements were drawn from 123 patients, none of whom used cyclooxygenase inhibitors or corticosteroids.

Blood samples. Blood was drawn from the antecubital vein and collected into three 4-mL endotoxin-free EDTA tubes. Body temperature and time of venipuncture were noted. Most blood was collected between 10 A.M. and 2 P.M. by one investigator (E.M.H.A.d.K.) and processed as described elsewhere [4].

Cytokine measurements. IL-1β, TNF-α, and IL-1ra were measured by RIA as described [4]. Interassay variation of the RIAs is <15%, and intraassay variation is <10%. The sensitivity of the assay with a 100-μL sample was 20 pg/mL for IL-1ra, IL-1β, and TNF-α. The TNF-α assay measures both free and soluble receptor-bound TNF-α. IL-6 was measured by ELISA (CLB, Amsterdam) according to the manufacturer's directions [5]. The lower limit of detection of the ELISA is 3 pg/mL. There is no interference of soluble (s) IL-6R. All samples from the same patient were analyzed in duplicate. Control values were determined from 20 healthy sedentary volunteers for IL-1β, IL-1ra, and TNF-α and from 50 healthy controls for IL-6. Median values (range) for circulating cytokines were as follows: IL-1β, 40 pg/mL (20–65); TNF-α, 92.5 pg/mL (65–120); IL-6, <3 pg/mL; and IL-1ra, 212.5 pg/mL (110–490). Medians (range) for cytokine ex vivo production were

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Informed consent was obtained from all patients, and the study was approved by all university hospital ethics committees.

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IL-1β, 6.5 ng/mL (2.2–15.5); TNF-α, 6.8 ng/mL (1.8–9.7); IL-6, 17.7 ng/mL (4.8–33.5), and IL-1ra, 10.8 ng/mL (7.2–16.8).

Statistical analysis. The Kruskal-Wallis nonparametric, Dunn's multiple comparison, and Mann-Whitney tests were used for statistical comparison of the median values of groups of patients with different diagnoses and of controls. Spearman's rank test was used to assess correlations because of non-Gaussian distribution. Multivariate analysis (multiple regression) was used to evaluate the effect on cytokine levels with the following variables: diagnosis category, sex, age, time of venipuncture (before or after 1 P.M.), fever pattern (continuous or recurrent), number of days between start of fever and venipuncture, fever course after venipuncture (descending, increasing, or stable), body temperature at venipuncture, and hospital in which blood was obtained. We could not include normal values in the multiple regression analysis.

Results

Patient characteristics. The median age for the 123 patients was 52 years (range, 16–87). There were 61 males and 62 females. Seven patients died of the underlying disease causing the fever.

Circulating cytokines (figure 1). Median circulating IL-1β concentrations for all patients was 55 pg/mL (range, 20–150). Median IL-1β concentrations for all 5 diagnostic groups and controls differed ($P < .005$). Patients with neoplasms, infections, and noninfectious inflammatory diseases (NIID) had significantly higher median circulating IL-1β than control subjects ($P < .05$), but there were no significant differences between diagnostic groups.

Median circulating TNF-α concentrations for all patients with FUO was 110 pg/mL (range, 20–510). Patients with infections had significantly higher median circulating TNF-α than controls ($P < .01$). There were no differences between diagnostic groups.

Median circulating IL-6 concentration for all patients was not determined (range, 0–2190 pg/mL). Patients with malignancies, infections, and NIID had higher levels of IL-6 than normal controls ($P < .001$). Patients with malignancies had higher levels than those with no diagnosis or miscellaneous disorders ($P < .05$).

Median circulating IL-1ra concentration in all patients with FUO was 320 pg/mL (range, 0–2100). Patients with neoplasms, infections, and NIID had significantly higher median circulating IL-1ra than controls ($P < .01$). Patients with neoplasms had higher levels of IL-1ra than those with NIID ($P < .01$). By multiple regression analysis, some explanatory variance was found. Higher IL-1ra levels were associated with increasing age, presence of continuous fever, and lower body temperature. Multiple regression analysis also indicated that patients with infections and malignancy had higher levels of IL-1ra than patients with no diagnosis.

Ex vivo cytokine production (figure 2). The median ex vivo production of IL-1β for all patients with FUO was 8.4 ng/mL (range, 0–47.9). There were no significant differences between the subgroups and normal subjects. By multiple regression analysis, some explanatory variance was found: Older patients tended to produce more IL-1β, and venipuncture in the afternoon was associated with lower production.

The median ex vivo production of TNF-α in all patients with FUO was 4.2 ng/mL (range, 0–9.8). Patients with neoplasms and NIID ($P < .05$) had significantly lower ex vivo production of TNF-α than normal subjects. By multiple regression analysis, males had lower ex vivo production than females.

Median production of IL-6 in all patients with FUO was 10.9 ng/mL (range, 3–36.3). Patients with no diagnosis, malignancies, infections, and NIID had significantly lower median IL-6 levels than normal controls ($P < .05$). Multiple regression analysis indicated that patients without fever at venipuncture had less IL-6 than did patients with fever ($P = .02$).

The median ex vivo production of IL-1ra of patients with FUO was 11.4 ng/mL (range, 0–21.6). There were no significant differences among the 6 groups. By multivariate analysis, some explanatory variance was found: When the period between first day of fever and venipuncture was long, ex vivo production of IL-1ra was higher, and patients whose body temperatures rose after venipuncture had higher levels of IL-1ra.

We found positive correlations between some circulating cytokines: IL-1β and TNF-α ($r = .31$, $P < .001$) and IL-1ra and IL-6 ($r = .33$, $P < .001$). Stronger positive correlations were found for ex vivo production of IL-1β and TNF-α ($r = .70$, $P < .001$), IL-1β and IL-6 ($r = .65$, $P < .001$), and TNF-α and IL-6 ($r = .53$, $P < .001$).

There was negative correlation for all circulating cytokines and their ex vivo production, but this was significant only for IL-1ra ($r = -.53$, $P < .001$).

When we compared circulating and ex vivo production of cytokines of the 7 patients who died with levels for patients still alive, only median circulating IL-1ra differed significantly for those who died (950 pg/mL; range, 525–1550) and for surviving patients (310 pg/mL; range, 20–2100; $P < .001$).

In 35 of the 123 patients with longstanding fever, no increased circulating cytokines could be measured (18 of 39 without a diagnosis and 17 of 84 with diagnoses; $P < .005$).

Discussion

We believe this study is the first prospective investigation of the role of the putative pyrogenic cytokines in FUO. We measured concentrations and ex vivo production of cytokines and evaluated their diagnostic value. Taken together, no pyrogenic cytokines were responsible for the fever in many patients and we could not link certain pyrogenic cytokines with disease categories. Despite the wide scatter, patients with infections had high IL-1ra and TNF-α plasma levels, patients with neoplasms had high IL-1β and IL-1ra plasma levels and low ex vivo production of TNF-α, and patients with NIID had low production of TNF-α.

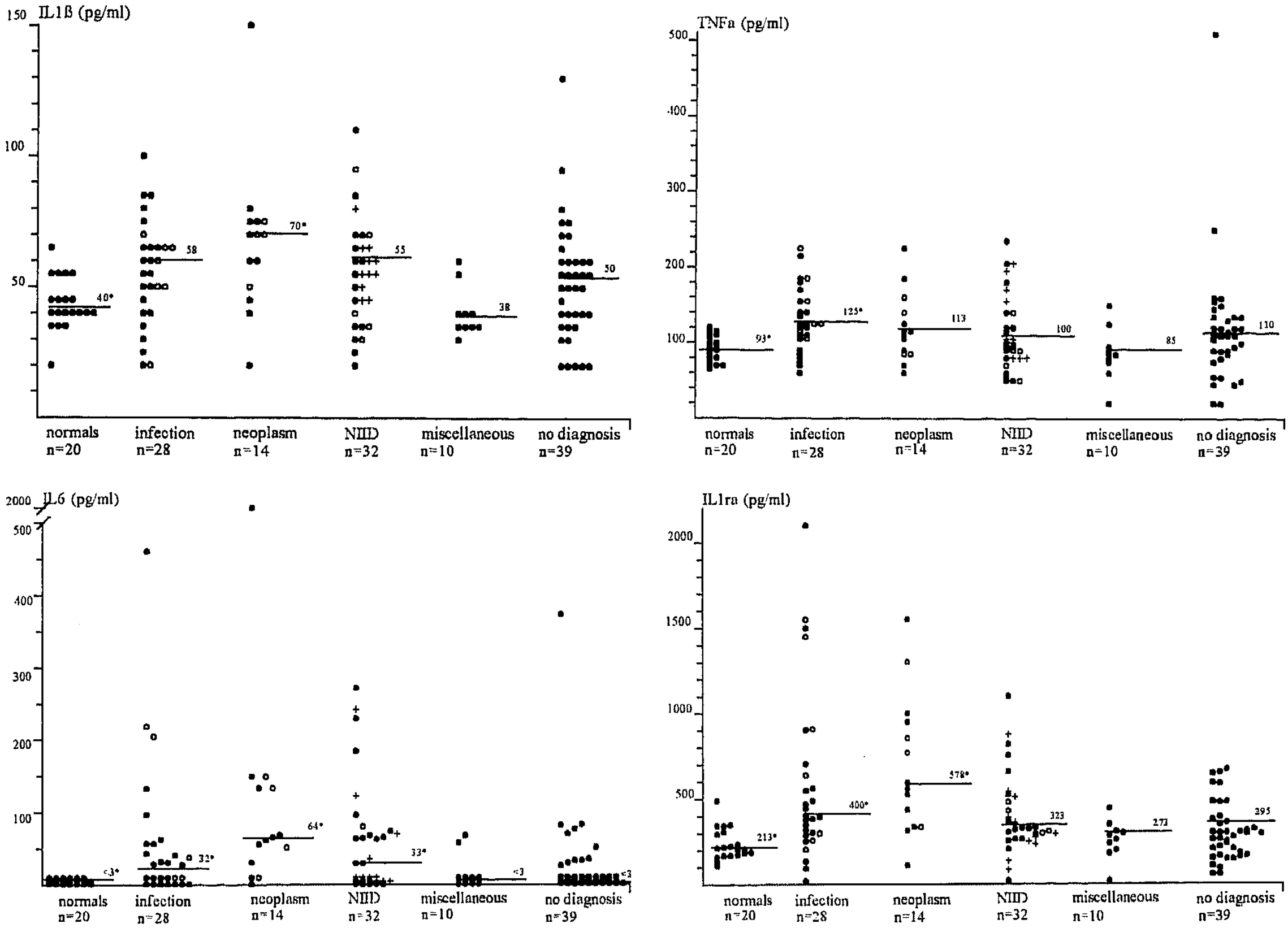

Figure 1. Circulating interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and IL-1ra (pg/mL) in 123 patients with FUO by diagnosis. Symbols indicate subgroups—bacterial infection, hematologic tumors, and vasculitis/mixed cryoglobulinemia (○); nonbacterial infection, solid tumors, and granulomatous diseases (●); connective tissue (+). Bars indicate medians. Nos. are median concentrations (pg/mL). * $P < .05$.

The possibility that cytokine patterns might be useful in the diagnostic work-up of a patient with FUO has been suggested. Patients with viral meningitis have lower concentrations of TNF-α in cerebrospinal fluid than do patients with bacterial meningitis [6]. Similarly, patients with granulocytopenia and proven bacterial infections have higher IL-6 concentrations than patients with FUO [7]. Appreciable concentrations of IL-1β and TNF-α have been found in patients with meningococcal disease [8] but not in patients with typhoid fever [9]. Variation in cytokine levels has been observed in other diseases, including chronic inflammatory bowel disease [10], rheumatoid arthritis [11], and Hodgkin's disease [12]. In our series of patients with FUO, despite significant differences in concentrations and ex vivo production capacity of cytokines and inhibitors between subgroups, these measurements did not aid in the diagnostic process. Pathophysiologic interpretation of the cytokine patterns found is difficult because of the degree of variation between patients with the same disease. It may well be that factors such as genetic background and nutritional status are predominant determinants in individual cytokine responses.

IL-1β, TNF-α, and IL-6 are thought to be the major endogenous pyrogens. Despite long-standing fever in all patients and a febrile state in most, we were unable to detect elevated concentrations of pyrogenic cytokines in all patients. There are a number of possible explanations for this phenomenon. Since the pyrogenic cytokines have a short half-life, it is possible that even if the set point in the hypothalamus had become elevated, the molecules had disappeared by the time of sampling. Alternatively, the set point may be raised by cytokines that are produced at the level of the organum vasculosum of the lamina terminalis [2]. These cytokines would then be induced by a circulating exogenous pyrogen or some endogenous molecule. Finally, there is the possibility that a major pyrogenic cytokine has yet to be identified.

Despite reports of increased concentrations of TNF-α in patients with fatal infection [13], TNF-α, IL-1β, and IL-6 concentrations were similar in patients who died of febrile illness

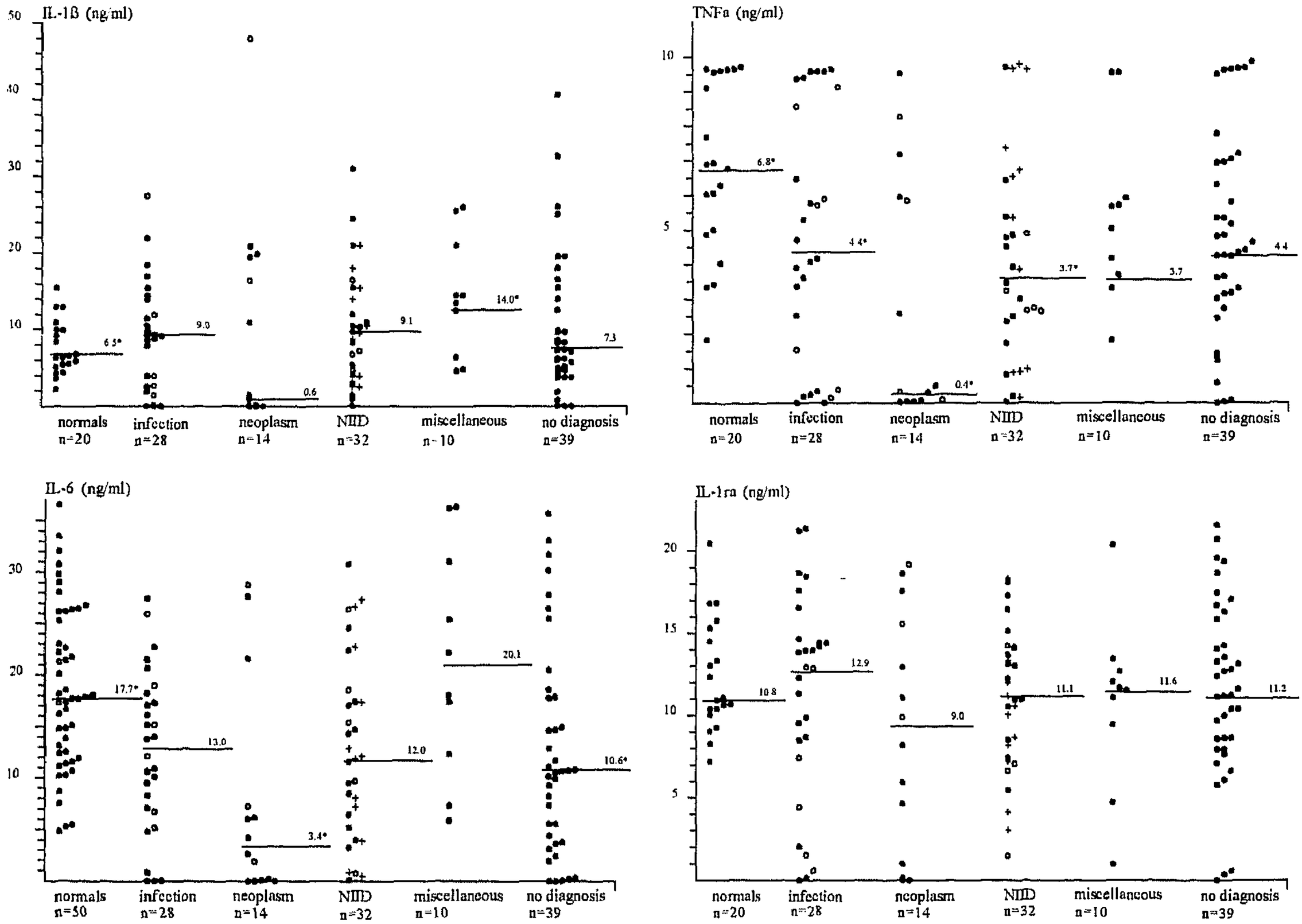

Figure 2. Ex vivo production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and IL-1ra (ng/mL) in 123 patients with FUO by diagnosis. Symbols indicate subgroups—bacterial infection, hematologic tumors, and vasculitis/mixed cryoglobulinemia (○); nonbacterial infection, solid tumors, and granulomatous diseases (●); connective tissue (+). Bars indicate medians. Nos. are median concentrations (pg/mL). * $P < .05$.

and in survivors. However, the median concentration of the antiinflammatory cytokine IL-1ra in those who died was higher than in surviving patients, pointing to greater cytokine activation in the former group.

In conclusion, this prospective study shows subtle changes in circulating concentrations and in ex vivo production of cytokines associated with FUO, but the measurements did not help in the diagnostic process of the intriguing clinical problem of FUO.

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Diagnosis of Measles with an IgM Capture EIA: The Optimal Timing of Specimen Collection after Rash Onset

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The optimal timing for collection of a single serum specimen to diagnose measles by using a monoclonal antibody–capture EIA was evaluated. Results of testing paired serum samples from 166 measles cases with at least 1 IgM-positive specimen were analyzed. Among persons whose second samples were IgM-positive, the seropositivity rate for first samples was 77% when collected within 72 h and 100% when collected 4–11 days after rash onset. Among unvaccinated persons whose first samples were IgM-positive, the rate for IgM positivity of second specimens declined from 100% at 4 days to 94% at 4 weeks after rash onset, then declined further to 63% at 5 weeks. Some previously vaccinated persons became IgM-negative during the third week after rash onset. In general, a single serum specimen collected between 72 h and 4 weeks after rash onset can be used to diagnose most cases of measles with an IgM capture EIA.

Measles continues to be a major health problem worldwide, with ~45 million cases globally each year [1]. The Pan American Health Organization is working toward the elimination of measles from Central and South America [2]. These elimination efforts have created renewed interest in sensitive and specific diagnostic assays that can be used by countries throughout the world to diagnose measles infections.

Currently, many different serologic techniques are used to diagnose measles; these are based on detecting either IgM or IgG antibodies [3, 4]. Serologic assays that detect IgG antibodies, including hemagglutination inhibition, RIA, plaque reduction neutralization, and microneutralization, have the disadvantage of requiring acute- and convalescent-phase specimens to measure a rise in IgG antibodies. Assays that detect IgM antibodies, such as RIAs and EIAs, often can be used to diagnose measles by testing only a single serum specimen. RIAs are reliable but have the disadvantage of requiring adequate facilities to store, use, and dispose of radioactive material. Most commercially available EIA kits use an indirect format. Although this format is relatively simple to use, its increased risk of false-positive results [5] and lower sensitivity can lead to misclassification of individual cases and outbreaks of measles-

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