The assessment of past chronic lead exposure is difficult. Chronic lead burden is not always correctly assessed using laboratory-based tests that are useful for acute or recent exposures. We describe a case of suspected chronic lead exposure that illustrated the need for improved and possibly noninvasive methods to determine cumulative lead body burden. X-Ray fluorescence (XRF) is discussed as a method to obtain in vivo bone lead measurements. We discuss the potential of such measurements as accurate biomarkers of cumulative exposure and whether XRF can be used for retroactive exposure assessment or to predict risk of future health problems.

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Case

A 53-year-old male was referred to a neurologist with an 8-year history of numbness in his hands and feet that was originally diagnosed as carpal tunnel syndrome. He complained of progressive weakness in his hands and pain in his elbows, neck, and shoulders. Exam findings included a mild peripheral neuropathy involving both motor and sensory components. A loss of pain sensation in the hands and feet as well as a loss of vibratory sensation in the toes was demonstrated. The patient claimed that his reading speed and language skills began to regress in his mid-20s. Since then he had complained of slowed thinking and had experienced some memory loss and mild dementia. The patient also had a 14-year history of hypertension, but he did not smoke or drink. Other conditions that could cause cognitive defects together with peripheral neuropathy, including stroke, Alzheimer disease, vitamin E or B12 deficiency, and hypothyroidism, were ruled out.

Between the ages of 22 and 42, the patient was employed at a lead smelter. During the course of his employment, blood lead values were monitored periodically and on more than one occasion were high enough to warrant temporary removal from the work site. Although the records are unavailable, the blood lead values may have approached or exceeded the maximum permissible occupational exposure limits, which at that time were 3.5-4 \( \mu \text{mol/L} \) (70–80 \( \mu \text{g/dL} \)) (1). It was felt that his medical history and current symptoms could have been caused by occupational exposure to lead.

Because the exposure had ended 12 years previously, the usual laboratory measurements of recent lead exposure were unlikely to be useful in the assessment of his total lead body burden (see below). Indeed, blood lead values were within the reference interval at 0.25 \( \mu \text{mol/L} \) [5 \( \mu \text{g/dL} \), reference interval <1 \( \mu \text{mol/L} \) (<20 \( \mu \text{g/dL} \)], and urine lead was undetectable [<0.25 \( \mu \text{mol/L} \) (<5 \( \mu \text{g/dL} \) h); reference interval, 0–4 \( \mu \text{mol/L} \) (0–80 \( \mu \text{g/dL} \) h)]. Blood lead concentrations measured before admission were normal and were not repeated in light of the urine lead findings. Protoporphyrin measurements were not performed. Because lead accumulates in the bone where it remains for long periods of time, the laboratory was contacted to investigate measurements of lead in bone or other means to determine past exposure.

Lead Health Risks, Metabolism, and Storage

Lead exposure causes numerous health problems, affecting nearly every system of the body (2, 3). As laboratory and epidemiological research into lead toxicity has progressed, evidence has accumulated associating chronic low-level lead exposure with a variety of health problems (1, 3, 4). Because lead is especially toxic to the developing central nervous system of young children (3), considerable attention has been given to the problem of early childhood lead exposure. Early studies that focused mainly on children with known histories of high lead exposure reported associations between increased blood lead concentrations and impairment of fine motor skills as well as a variety of neurobehavioral and cognitive defects, including behavioral problems and decreased IQ (1, 5, 6). Particular concern over the effect of lead on IQ spurred several large-scale prospective studies. Blood lead con-
centrations were monitored from birth through school age, and children were evaluated using a variety of standardized IQ tests designed for children. Results were controlled for socioeconomic status, maternal IQ, and other factors that correlate with IQ. A meta-analysis of these studies concluded that an increase in blood lead concentrations from 0.5 to 1 μmol/L (10 to 20 μg/dL) lowered IQ by 2.2 points (7). These and other studies led to the initiation of federally mandated lead screening programs. In addition, the maximum permissible lead concentration for children has been progressively lowered by the Centers for Disease Control and Prevention from 2 μmol/L (40 μg/dL) in the early 1970s to the current action concentration of 0.5 μmol (10 μg/dL) (1). More recently, chronic subclinical lead exposure has been linked with several health problems in adults, including anemia (8), chronic progressive renal dysfunction (4, 9, 10), peripheral neuropathy (3, 4, 11), hypertension (12), and various reproductive problems (3, 4, 13). Acute lead poisoning, characterized by persistent high blood lead concentrations [4 μmol/L (80 μg/dL) in children, 4–5 μmol/L (80–100 μg/dL) in adults] can lead to renal failure, encephalopathy, and death.

The total body burden of lead in 20th century humans peaked at concentrations ~1000-fold higher than those measured in pre-industrial society (14). Industrial activity has increased the environmental availability of lead. Although use of lead paint and leaded gasoline has been largely discontinued, leading to markedly lower population blood lead values (15, 16), substantial amounts of lead have accumulated in the soil and dust near roadways and lead-painted homes. The resulting lead-containing dust can be inhaled and absorbed through the lungs or ingested and absorbed through the gastrointestinal tract. Lead is excreted primarily through the feces but also in the urine and, to a very minor extent, in sweat and saliva (3). Because lead excretion is rather inefficient, most of an absorbed lead dose will be stored in the body.

As shown in Fig. 1, there are three main compartments where lead distributes: blood, soft tissue, and bone. After being absorbed from the lungs or gastrointestinal tract, lead first enters the red blood cells (RBCs),3 where it displaces zinc from the active site of various hematopoietic enzymes. Blood lead represents only 1–5% of the total body burden of lead (4, 17). At lower lead concentrations, 95–99% of blood lead is bound to RBCs and only 1% is found in the ionized form in plasma. At higher concentrations, the binding sites in the RBCs become saturated and more lead can be found in the plasma. Plasma lead can easily exchange into soft tissues, primarily the kidney and brain, where it exerts its most toxic effects. It binds to cell membranes, alters protein structure, and may interfere with gene translation (2). Plasma lead is also available for exchange into bone mineral, where lead replaces the calcium in hydroxyapatite crystals (18).

Bone lead comprises 70–80% of the total body burden of lead in children and 90–95% of the total body burden in adults (3, 7). Because of bone’s relatively slow metabolic turnover, deposited lead persists for years, and bone lead stores accumulate slowly throughout life; therefore, bone lead may represent a more accurate cumulative biomarker of lead body burden than either blood or soft tissue lead.

Bone lead acts as an endogenous source of lead, and by continuous release to other tissues it can represent a health risk long after exposure has ended. Release of lead from the bones will increase in settings of increased bone turnover, such as osteoporosis, pregnancy, lactation, and certain hyperendocrine states. Indeed, there have been reports in the literature of acute lead poisoning (in the absence of recent exposure) associated with hyperthyroidism (19, 20), and skeletal lead release has been quantitatively demonstrated in pregnant and lactating women (21). Even in normal metabolic states, slow release of lead from bone can maintain chronically increased blood lead values in a heavily exposed individual long after external exposure has ceased (22). In light of this observation, several studies have been aimed at determining whether bone lead constitutes an independent risk factor for conditions associated with chronic lead exposure.

**Methods for Measuring Lead Body Burden**

The usual clinical laboratory-based methods for assessing lead exposure include direct determinations of lead in blood and in urine as well as measurements of various components of the heme biosynthetic pathway, which is inhibited by lead. To guide their treatment decisions, clinicians ultimately rely on whole blood lead values, which are quantified by atomic absorption-based or voltammetric methods (23). Because of the sensitivities of these methods and the fact that lead is ubiquitous in the environment, especially in dust particles, great care must be taken to avoid contamination during sample collection to avoid false positives (23). Special blood collection tubes designed specifically for trace metal analysis should be used in preference to other tubes (23).

Measurements of the heme precursors free erythrocyte protoporphyrin (FEP) and erythrocyte zinc protoporphyrin (ZnP) have also been used for screening purposes. Because these heme precursors are not sensitive indicators of blood lead concentrations <1.25 μmol/L (25 μg/dL) (24) and can be increased in other conditions, such as iron deficiency (23), blood lead measurements remain the preferred screening tool. Increased concentrations of FEP or ZnP denote impairment of the heme biosynthetic pathway and therefore may indicate a more prolonged recent exposure to lead. From a practical point of view, FEP and ZnP measurements are less prone to sample contamination and can be performed using a

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3 Nonstandard abbreviations: RBC, red blood cell; FEP, free erythrocyte protoporphyrin; ZnP, zinc protoporphyrin; XRF, x-ray fluorescence; and MDL, minimum detectable level.
relatively inexpensive hematofluorometer (3, 23, 25), but because of the finite lifetime of affected erythrocytes and the resulting short half-life of lead in blood, these laboratory-based measurements can assess only ongoing or recent lead exposure, within a period of a few weeks to months.

If longer-term exposure is suspected, a somewhat more accurate picture of the overall lead body burden can be provided by the provocative chelation test (3, 26, 27). After administration of a chelating agent such as calcium disodium ethylenediaminetetraacetic acid (CaNa₂EDTA), lead is mobilized from the soft tissues and excreted in the urine, where it is quantified by atomic absorption (2, 3). Increased urine lead concentrations signify prolonged lead exposure and indicate the need for additional treatment. Determination of lead in hair has also been considered for assessment of chronic exposure, but variability attributable to differing hair types and textures, as well as the high possibility of external contamination from dust-borne lead, can render such measurements difficult to interpret (7, 17).

True assessment of cumulative lead body burden should use measurements of bone lead, where most of the body lead burden is concentrated. Historically, such measurements involve wet chemical digestion of bone material followed by atomic absorption-based measurements and were limited to specimens from cadavers or bone biopsy samples. Measurement of tooth lead is possible by similar means. Like bone lead, tooth lead increases as a function of age and exposure (7, 28, 29). Such measurements have been widely performed on shed primary teeth to assess lead exposure in children (6, 30). Measurements of lead in the permanent teeth of adults are less practical because of the highly invasive nature of sample collection, and are rarely performed.

**Bone Lead by X-Ray Fluorescence**

Over the past two decades, the technique of x-ray fluorescence (XRF) has emerged as a noninvasive method for bone lead determination, enabling direct in vivo measurements of skeletal lead (4, 17, 31). XRF occurs when absorption of a high-energy photon by a heavy metal atom induces the emission of a second x-ray photon of slightly lower energy. This fluorescent photon has an energy characteristic of the heavy metal atom from which it was ejected. A typical x-ray fluorometer designed for in vivo measurements features a radioactive ¹⁰⁹Cd source, which emits at 88.035 keV, and a germanium crystal detector arranged in backscatter geometry (i.e., the detector is mounted behind the source). During a typical in vivo bone lead measurement, the bone is irradiated for 30–60 min, and the generated photons are collected and counted. The experimental setup and resulting spectrum are shown in Fig. 2. In addition to the characteristic peaks resulting from specific electronic transitions in lead atoms, a large peak at the energy of the incident radiation is evident. This peak results from elastic scattering of the incident radiation by calcium atoms in the bone sample and thus is indicative of the bone mineral density. The first step in extracting the lead information is to curve-fit and subtract the heavy Compton background signal that is typical of XRF experiments. The area under one of the lead peaks, usually the Pb K-alpha 1 transition at 74.4 keV, is then measured and normalized to the area under the elastic scatter peak. This yields a measurement of bone lead in units of μg lead/g of bone mineral.

Calibration of x-ray fluorometers is accomplished using lead-doped plaster of Paris phantoms. The accuracy of the method has been established through comparison of XRF data with results from chemical analysis of specimens from cadavers; in a representative study a linear regression slope of 1.02 and a correlation coefficient of 0.98 were obtained (32). The sensitivity and precision of the method present more of an analytical challenge. In addition to the sample-to-sample reproducibility, which has been studied using repeated measurements of lead-doped phantoms (33), there is also a certain amount of imprecision associated with each calculated bone lead value. This uncertainty, estimated using goodness-of-fit statistics from the curve-fit of the background, can range...
from 3 to 30 μg lead/g of bone and can be problematic for low-level lead measurements (11, 17, 31, 34, 35). Treatment of low-level data varies depending on whether they are intended for use in a clinical setting or for epidemiological research. Standard clinical practice mandates calculation of a minimum detectable level (MDL) based on standard deviations of the background counts. When interpreting low-level results for clinical purposes, any measurement falling below the MDL is considered to be zero. The MDL varies from instrument to instrument, but it is typically ~3–15 μg lead/g of bone mineral. Because the bone lead concentrations for young or unexposed patients may not be much higher than this (see Table 1), clinical interpretation of XRF results for these individuals may be problematic. Many epidemiologists prefer to use all estimates generated by the instrument, including even negative values for bone lead (17, 34, 36). Rejecting or recoding measurements that fall below an instrument’s MDL can yield results that are artificially biased (34). Therefore, in epidemiological research, each bone lead value is used along with its individual uncertainty to calculate and compare population means and medians.

Each bone lead determination relies on the accumulation of a large number of data points, and more photon counts will lead to better background curve fitting and less imprecision. Experimental conditions affecting photon count include the activity of the photon source, the efficiency of the detector, and the measurement time. Higher photon yield will be obtained with large, dense, well-mineralized bones containing more calcium and lead. Photon scattering by overlying tissue or movement of the subject during the sampling period can decrease the photon count. Normalizing the lead signal to the calcium signal ensures that the accuracy of the measurement is not affected by these variables. However, the need to accumulate a large number of data points has certain implications for sampling and renders XRF measurements difficult.

### Table 1. Tibia lead in various populations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tibia lead, μg/g bone mineral</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suburban teens</td>
<td>4</td>
<td>(36)</td>
</tr>
<tr>
<td>Women of childbearing age</td>
<td>10</td>
<td>(45)</td>
</tr>
<tr>
<td>Adults with history of childhood lead poisoning</td>
<td>22</td>
<td>(11)</td>
</tr>
<tr>
<td>Age-matched controls</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>65-year-old males</td>
<td>22</td>
<td>(12)</td>
</tr>
<tr>
<td>Active lead workers</td>
<td>21</td>
<td>(39)</td>
</tr>
<tr>
<td>Retired lead workers</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Lead factory office workers</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Unexposed controls</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Lead workers</td>
<td>25</td>
<td>(35)</td>
</tr>
<tr>
<td>Unexposed controls</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lead workers</td>
<td>48</td>
<td>(27)</td>
</tr>
<tr>
<td>Lead workers</td>
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<td>(42)</td>
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<tr>
<td>Lead workers</td>
<td>66</td>
<td>(40)</td>
</tr>
</tbody>
</table>
cult in certain populations. For example, XRF measurements are less precise in obese subjects and in children with smaller, less well-mineralized bones.

These considerations also affect the choice of bone for XRF measurements. From a sampling point of view, the best bone to use is a large dense bone with very little overlying tissue; therefore, the tibia is usually preferred for XRF measurements. However, most bones are a mixture of two different bone types, cortical and trabecular, which represent distinct metabolic compartments. Trabecular bone, because of its higher surface area, has a faster rate of turnover than denser cortical bone. The behavior of lead in each type of bone may also differ; therefore, some researchers have chosen to measure bone lead in two sites. The tibia and the phalanx are examples of primarily cortical bone, whereas the patella and calcaneus are considered mainly trabecular. Although bone lead reference intervals have not been rigorously established, Table 1 shows some typical values of tibia lead obtained using XRF in various populations.

**Bone Lead as a Cumulative Exposure Marker**

The utility of bone lead as a marker of cumulative lead exposure has been established by studying populations of heavily exposed lead workers. As part of an occupational exposure monitoring program, these workers had blood lead concentrations measured and recorded throughout their employment. A time-integrated cumulative blood lead index was calculated from the recorded blood lead values and correlated to measurements of bone lead (35, 37–39). The observed correlation coefficients for four of these studies ranged from 0.67 to 0.88 (35, 37–39). Fig. 3 depicts data from one study (38).

The cumulative time-integrated blood lead index reflects both the duration and intensity of lead exposure. Erkkilla et al. (39) showed that bone lead concentrations in the two separate metabolic compartments are affected differently by these two variables. A time-weighted average blood lead was calculated to reflect the intensity of the exposure, and bone lead was measured in the tibia and calcaneus of 91 active lead workers. It was found that the tibia lead concentration is significantly related to both the duration and the intensity of exposure, whereas lead in the trabecular calcaneus bone reflects only the intensity. This suggests that trabecular bone lead represents a relatively rapidly exchangeable bone lead compartment, in contrast to cortical lead.

**Bone Lead as a Retroactive Exposure Marker**

In determining whether bone lead concentrations can be used retroactively to assess prior exposure to lead, it is necessary to understand what happens to bone lead concentrations after exposure ends. Nilsson et al. (22) followed a group of 14 lead workers for >18 years after retirement with measurements of lead in the blood and the phalanx. The blood lead concentrations experienced an initial rapid decline after the end of exposure and then continued to decrease slowly over a period of years. A tri-exponential model was used to describe this decrease, yielding half-lives of 34 days, 1.2 years, and 13 years. The shortest half-life correlates well with the known half-life of lead in RBCs. The intermediate value is assumed to result from lead in soft tissues and the rapidly exchangeable trabecular compartment. The finger bone lead measurements decreased according to a mono-exponential curve with a calculated half-life of 16 years, which was in good agreement with the longest half-life from the blood lead measurements. Thus, the release of bone lead into blood can maintain increased blood lead concentrations for years after the end of occupational exposure.

A more complete model for retroactive exposure assessment, accounting for this endogenous release of bone lead into the bloodstream, was developed by Bergdahl et al. (35) and Borjesson et al. (37). They first mathematically modeled the flux of lead into and out of the body, and between the skeletal and central (blood) pools, under steady-state conditions. They then calculated several different values for a bone-to-plasma and an overall elimination half-life. These half-lives were then used to correct the cumulative blood lead indices of two separate groups of actively exposed lead workers. The resulting adjusted indices were then plotted against the concentration of bone lead. In the study by Borjesson et al. (37) of a group of 137 workers monitored with phalanx lead measurements, the best correlation was observed with an elimination half-life of 14 years. The study by Bergdahl et al.
yielded a half-life of 13 years, using determinations of tibial bone lead in 77 workers. With this information, a three-dimensional model such as the one shown in Fig. 4 can be prepared to describe the relationship among bone lead, blood lead, and exposure time. Assuming that exposure to lead has been constant, this model can be used to assess retroactive exposure if the current bone and blood lead concentrations are known. Although the usefulness of such a model is limited by its simplicity and its applicability only to the population from which it was derived, it illustrates one approach to estimate retroactive exposure.

**Bone Lead as a Risk Factor for Disease**

Because bone lead serves as a biomarker of cumulative lead exposure, it may be a better predictor of adverse health effects associated with chronic lead exposure. Several studies have been aimed at determining whether bone lead constitutes an independent risk factor compared with other biomarkers of lead exposure in certain disease settings. In general, these studies involved use of multivariate analysis and various regression techniques to search for statistically significant associations between bone lead and some determinant of disease condition.

At least two studies have looked for an association between renal dysfunction and bone lead concentrations in occupationally exposed populations of lead smelter workers (40, 41). In both of these studies, bone lead concentrations indicated a threefold higher body burden of lead compared with unexposed controls, but no indicators of early tubular or glomerular damage were increased. The study by Roels et al. (40) did find a positive correlation between tibia lead and creatinine clearance, suggesting that lead exposure may be associated with hyperfiltration after an oral protein load. However, no correlation was found between markers of lead exposure and kidney damage. In fact, the more sensitive indicators of early tubular and glomerular damage (such as urinary microglobulin and N-acetyl-β-glucosaminidase) were unchanged between the lead-exposed group and controls. Although chronic low-level lead exposure is known to be associated with nephropathies (9), studies using bone lead provide no evidence that occupational exposure causes irreversible kidney damage.

Several studies have examined whether bone lead can be used to establish a link between lead exposure and neurotoxicity. Bleecker et al. (42) evaluated 80 active lead smelter workers with six standardized tests of verbal memory and visuomotor function. Compared with blood lead indices (including current, cumulative, and time-weighted average blood lead), tibia lead was a poor predictor of performance, being significantly associated with only one of these tests (the Grooved Pegboard test). Two hundred eighty-one young adults with a history of childhood lead poisoning had altered tests of peripheral nervous function and neurobehavioral function compared with age-matched controls (11). However, tibia lead did not correlate significantly with any of the 26 test outcomes. High tibia lead was found to be associated with increased reports of delinquency, aggressive behavior, and other problems in a study of 301 11-year-old boys assessed using the Child Behavior Checklist (43). The

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Fig. 4. Three-dimensional model showing the relationship among bone lead (Bone-Pb), concurrently obtained blood lead (B-Pb), and exposure time under the conditions of constant lead exposure. Reproduced from Archives of Environmental Health 1997; 52:104–12 (37), with permission from The Helen Dwight Reid Educational Foundation, Heldref Publications.
The association was not as strong as that found in another study using lead concentrations in shed primary teeth (30). Studies of this nature are limited by the inherently subjective nature of neuropsychological tests (44), as well as the imprecision of bone lead determinations, especially in younger subjects.

Some studies have found bone lead to be a better biomarker of lead exposure than blood lead concentrations for predicting certain forms of toxicity. A correlation between patella lead and decreased hemoglobin and hematocrit was found in a study of 116 moderately lead-exposed construction workers (8). Tibia lead was associated with increased risk of hypertension in a study of 590 elderly men (12). A study of 272 mother-infant pairs from Mexico City, an area with considerable environmental lead exposure, demonstrated decreased infant birth weight associated with high maternal tibia lead values (45).

Blood lead values were not significantly associated with adverse outcomes in any of these three studies, indicating that chronic and not recent exposure is responsible.

**Conclusion**

In summary, XRF measurements of bone lead provide a noninvasive measurement of total lead body burden. Studies of occupationally exposed workers have established bone lead as a biomarker of cumulative exposure. In some settings, bone lead concentrations can be used to estimate retroactive blood lead concentrations. Although some evidence suggests that bone lead may be a risk factor in various disease states associated with chronic lead exposure, definitive correlations have not been demonstrated. Some studies do suggest that bone lead may provide information beyond that given by laboratory-based measurements of lead status. For example, studies with pregnant and lactating women suggest a possible role for screening bone lead in women of childbearing age with prior history of lead exposure to determine the need for chelation therapy or calcium supplementation. Many questions remain, and more research is needed before the clinical utility of bone lead measurements can be fully assessed. Only ~12 institutions in North America are equipped to perform in vivo bone lead measurements on a research basis. Whereas continued technical improvements in instrumentation should eventually facilitate such measurements, the clinical utility of bone lead measurements currently is limited by experimental imprecision and poor availability.

**Case Report**

XRF bone lead measurements were deemed impractical in this patient because of the need for considerable travel, expense, and inconvenience. Two of the patient’s teeth, removed for dental health purposes, had lead concentrations of 34.5 μg/g, which is well above the analysis laboratory’s quoted age-independent reference limit of <10 μg/g. Although certainly suggestive of an increased lead body burden, this measurement is difficult to interpret in the absence of well-defined age-specific reference intervals. Various reports have clearly demonstrated that the concentration of tooth lead increases with age (7, 28, 29), and values reported for average tooth lead in a 50-year-old man range from 15 to 50 μg/g (28, 29, 46).

Although a markedly increased total lead body burden was not conclusively demonstrated in this patient, it was nevertheless strongly felt that lead exposure had played a significant role in his health status. Although usually reserved for cases of recent exposure where blood lead concentrations are demonstrably high, chelation therapy was considered as a means to remove lead from the body. This can be accomplished either through intramuscular injections of CaNa₂EDTA (3, 26, 27) or by administration of an oral chelating drug such as Chemet (2,3-dimercaptoposuccinic acid) (47). Patients with long-standing lead-induced nephropathy have shown slight improvements in renal function in response to prolonged EDTA chelation therapy (26, 48), but it is unknown whether chelation therapy would also benefit patients suffering from other manifestations of chronic lead exposure. A course of Chemet was prescribed for this patient, and his condition continues to be monitored.

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