Use of phlebotomy treatment in Atlantic bottlenose dolphins with iron overload

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Case Description—3 adult (24- to 43-year-old) Atlantic bottlenose dolphins (*Tursiops truncatus*) with chronic episodic malaise and inappetence associated with high serum aminotransferase (alanine aminotransferase and aspartate aminotransferase) activities, high serum iron concentration, and serum transferrin saturation > 80% were evaluated.

Clinical Findings—Results of histologic examination of liver biopsy specimens revealed hemosiderosis in all 3 dolphins. Except for chronic lymphocytosis in 1 dolphin, results of extensive diagnostic testing revealed no other abnormalities. For each dolphin, a diagnosis of iron overload of unknown origin was made.

Treatment and Outcome—Phlebotomy treatment was implemented to reduce body stores of iron. Each phlebotomy procedure removed 7% to 17% (1 to 3 L) of estimated blood volume. Treatment consisted of an induction phase of weekly phlebotomy procedures for 22 to 30 weeks, which was complete when serum iron concentration and aminotransferase activities were within reference ranges and serum transferrin saturation was ≤ 20% or Hct was ≤ 30%. Total amount of iron removed from each dolphin was 53 to 111 mg/kg (24.1 to 50.5 mg/lb) of body weight. One dolphin required maintenance procedures at 8- to 12-week intervals when high serum iron concentration was detected.

Clinical Relevance—Although the cause of the iron overload and high serum aminotransferase activities remained unknown, phlebotomy treatment successfully resolved the clinicopathologic abnormalities, supporting a role of iron overload in the hepatopathy of the 3 dolphins. (*J Am Vet Med Assoc* 2009;235:194–200)

Three Atlantic bottlenose dolphins (*Tursiops truncatus*) cared for by the United States Navy Marine Mammal Program had extended (13- to 19-year) histories of chronic, episodic elevations in serum ALT and AST activities and iron concentration, compared with sex- and age-adjusted reference ranges[1]; the reference ranges were established among healthy dolphins in the same population. The 3 dolphins included dolphin A, a 24-year-old 197-kg (433-lb) female; dolphin B, a 29-year-old 247-kg (543-lb) male; and dolphin C, a 43-year-old 243-kg (535-lb) male. These 3 dolphins were among 6 bottlenose dolphins for which high serum ALT and AST activities and potential liver disease were recently described.[2] During 13 to 19 years of observation, the 3 dolphins had episodes of nonspecific clinical illness, such as malaise and inappetence. Attempted treatments included administration of antimicrobials with or without corticosteroids. Despite short-term resolution of clinical signs, episodic increases in serum ALT and AST activities and iron concentration continued over time in all 3 dolphins.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ESR	Erythrocyte sedimentation rate
GGT	γ-Glutamyltransferase
LDH	Lactate dehydrogenase
MCV	Mean corpuscular volume
PAVR	Periarterial vascular rete

The dolphins received supplemental vitamins and minerals daily and preventative antiparasitic medication biannually. Prior to 2003, iron was included in the mineral supplement. Starting in 2003, another supplement was administered that did not contain iron. Annual physical examination of each dolphin included a CBC, serum biochemical analyses, floatation examination of a fecal specimen, cytologic examination of swabbed specimens from the blowhole, forestomach gastroscopy, thoracic and abdominal ultrasonography, and thoracic radiography. Ultrasonography of the liver of each dolphin revealed no obvious abnormalities, compared with findings in other dolphins in the population; however, standardization of liver ultrasonographic techniques in dolphins has not been established. Ultrasound-guided liver biopsy specimens were obtained from each dolphin by use of a core biopsy needle[a] for histologic examination and other diagnostic tests.

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In 2001, histologic examination of liver tissue obtained from dolphin A revealed subacute to chronic mild periportal hepatitis and hemosiderosis. Histologic examination of sections stained with H&E and Perl's iron stains revealed mild periportal fibrosis and abundant iron pigment within the hepatocytes and sinusoidal macrophages. Dolphin B underwent a biopsy procedure in 2001, and histologic examination of liver tissue revealed chronic active hepatitis with hemosiderosis with abundant iron within the hepatocytes and sinusoidal macrophages. Histologic examination of sections stained with H&E, Gomori methenamine silver, and Gram stains failed to reveal any evidence of infectious agents. In 2007, histologic examination of liver tissue obtained from dolphin C revealed hemosiderosis with mild amounts of hepatocyte granular intracellular iron and moderate amounts of iron in the Kupffer cells. Results of bacterial and fungal cultures and viral isolations of the liver biopsy specimens from all 3 dolphins were negative.

Serum bilirubin concentration remained within the reference range (0.1 to 0.4 mg/dL) for each dolphin during the extended observation period. Other hepatic function tests, such as measurement of serum bile acids and blood ammonia concentrations, have not been validated in dolphins, and reference ranges for these variables are unknown; therefore, these tests were not performed. Except for a persistent mild lymphocytosis of unknown origin in dolphin A, no other underlying disease processes were identified in any of the dolphins.

The clinicopathologic and histologic findings without identification of an underlying cause led to the diagnosis of iron overload in all 3 dolphins. Phlebotomy treatment was implemented for each dolphin to reduce total body stores of iron and resolve the clinicopathologic abnormalities and clinical signs associated with iron overload.

Prior to each phlebotomy procedure, the dolphins were not fed for at least 15 hours to reduce the effects of feeding on the values of serum biochemical variables; alterations induced by feeding were previously identified in healthy bottlenose dolphins.[3] The dolphins were trained to voluntarily present the ventral aspect of their fluke or slide out of the water onto a foam-padded mat. In these positions, the peduncle PAVR was accessible for phlebotomy.[4] The phlebotomy site was aseptically prepared by use of povidone-iodine solution and isopropyl alcohol prior to venipuncture. Blood was collected by use of a specialized blood collection set[b] that consisted of tubing with a preattached IV needle (17 gauge; 1 and 7/8 inches in length) and a rubber piercing needle (15 gauge). The collection set was primed with 5 mL of heparin (1,000 U/mL) to prevent clotting, and blood was collected into 1- or 2-L evacuated bottles.[c] Blood samples for CBC, serum biochemical analysis, and measurement of total iron-binding capacity (for determination of serum transferrin saturation) were obtained at the end of each procedure. The duration of each phlebotomy procedure was 10 to 60 minutes. The dolphins were returned to a water enclosure after each procedure was concluded and monitored for 30 minutes. After each procedure, training was restricted to low-energy activities for 24 hours.

During the induction phase, the frequency of phlebotomy procedures and the volume of blood removed during each procedure were adapted from treatment guidelines for humans with hemochromatosis (iron storage disease).[5] Initially, 7% to 11% of the estimated blood volume was removed during each procedure. The estimated blood volume of bottlenose dolphins is 71 mL/kg (32.3 mL/lb) of body weight (7.1% of body weight).[6] Therefore, at the start of the induction phase, 5.1 to 8.2 mL of blood/kg (2.3 to 3.7 mL/lb) of body weight (total volume, 1 to 2 L) was removed from each dolphin during each procedure. A CBC and serum biochemical analysis were performed 24 hours prior to each subsequent phlebotomy procedure. If the Hct was ≤ 30% but the serum iron concentration and ALT and AST activities were not within reference limits, phlebotomy procedures were delayed until the Hct was > 30%. If no adverse effects were detected following the preceding phlebotomy procedure and an increased rate of erythropoiesis was evident on the basis of CBC findings (ie, a detectable increase in reticulocyte count [reference range, 1% to 2.3%], an increase in MCV [reference range, 117 to 137 fL], and presence of ≥ 2 nucleated RBCs/100 WBCs [reference range, 0 to 1 nucleated RBCs/100 WBCs]), the volume of blood removed during the next phlebotomy procedure was increased.

For each dolphin, the induction phase of phlebotomy treatment was completed when serum iron concentration was within the reference range (92 to 300 µg/dL); serum ALT and AST activities were within reference ranges (ALT, 13 to 54 U/L; AST, 118 to 398 U/L); and serum transferrin saturation was ≤ 20% or Hct was ≤ 30%. Among the dolphins, the duration of the induction phase of phlebotomy treatment was approximately 22 to 30 weeks (142 to 214 days).

Phlebotomy procedures were performed weekly, during each of which 7% to 17% (1 to 3 L) of the estimated blood volume was removed. Iron comprises 0.34% of hemoglobin. Therefore, the amount of iron removed during each phlebotomy procedure was calculated by use of an equation as follows:

Amount of iron (g) = hemoglobin concentration (g/dL) × 0.0034 × volume of removed blood (dL)[7]

During the 2-year period prior to commencement of phlebotomy treatment, results of repeated (n = 34) CBCs and serum biochemical analyses for dolphin A indicated a persistent mild lymphocytic leukocytosis (mean WBC count, 12,013 cells/µL [reference range, 4,275 to 11,336 cells/µL]; mean lymphocyte count, 2,978 cells/µL [reference range, 270 to 2,559 cells/µL]), high serum globulin concentration (mean value, 3.5 g/dL; reference range, 2.1 to 3.1 g/dL), and high ESR (mean value, 36 mm/h; reference range, 0 to 18 mm/h). Serum concentration of iron and activities of ALT, AST, GGT, and LDH were also elevated (**Table 1**). During the 2 months prior to phlebotomy treatment, prednisolone (0.2 mg/kg [0.09 mg/lb], PO, q 24 h) and doxycycline (5 mg/kg [2.3 mg/lb], PO, q 24 h) were administered to dolphin A because of clinical signs of generalized malaise and inconsistent appetite; additionally, high serum ALT, AST, GGT, and LDH activities were detected

Table 1—Least squares mean values of selected serum biochemical variables in 2 adult Atlantic bottlenose dolphins with iron overload determined during a 2-year period before initiation of an induction phase (preinduction period) and the initial 6-month period of a postinduction maintenance phase of phlebotomy treatment to illustrate the effectiveness of treatment.

		Dolphin A		Dolphin B	
Variable	Reference range	Preinduction period	Initial maintenance period*	Preinduction period	Initial maintenance period*
No. of samples evaluated	—	34	20	38	23
Iron (µg/dL)	92–300	771	220	615	151
ALT (U/L)	13–54	133	32	139	44
AST (U/L)	118–398	688	187	832	161
GGT (U/L)	21–48	407	25	893	47
LDH (U/L)	270–494	752	416	594	338
Transferrin saturation (%)†	NK	85	49	83	40

The duration of the induction phase of phlebotomy treatment was 149 days for dolphin A and 165 days for dolphin B. Dolphins A and B underwent 22 and 25 phlebotomy procedures, respectively, during the induction phase and 3 and 0 phlebotomy procedures, respectively, during the initial 6-month period of the maintenance phase. The data were adjusted for the interval between the previous meal and the time of sample collection prior to statistical analysis.

*For a given dolphin, the maintenance value differs significantly ($P < 0.001$) from the preinduction value for each variable. †For both the preinduction and maintenance periods, transferrin saturation was evaluated in 19 samples collected from dolphin A and in 20 samples collected from dolphin B.

— = Not applicable. NK = Not known.

episodically, and the serum iron concentration was progressively increasing, compared with previous values. As a result of treatment with prednisolone and doxycycline, the dolphin had signs of an improved overall attitude and the ESR and WBC count decreased; however, there was no decrease in serum iron concentration or ALT, AST, GGT, or LDH activity. Because of the lack of an effective response, administration of doxycycline was stopped 14 days prior to the start of phlebotomy treatment. Administration of prednisolone was continued as an effective appetite stimulant during initiation of phlebotomy treatment.

The induction phase for dolphin A included 22 phlebotomy procedures performed during a period of 22 weeks. Initially, 1 L of blood (approx 7% of estimated blood volume) was removed once a week for 3 weeks. The fourth procedure was delayed for 1 week to monitor response to the phlebotomy treatment, and then continued with removal of 1 L of blood once a week for 7 weeks. During the next 4 weeks, the volume of blood removed during each procedure was gradually increased to 2 L. Removal of 2 L of blood/procedure was continued until the induction phase was complete. There were gradual decreases in Hct and serum iron concentration, ALT and AST activities, and percentage of serum transferrin saturation after each phlebotomy procedure. After 4 weeks of phlebotomy treatment, the amount and frequency of prednisolone administration were gradually reduced, and administration of the drug was then discontinued because of signs of overall improvement in attitude and appetite. Increased erythropoiesis was evident: percentage of reticulocytes (4.86%) and nucleated RBC count (13 nucleated RBCs/100 WBCs) peaked at week 10 and remained elevated throughout the treatment period. Serum iron concentration was within the reference range after 9 weeks of phlebotomy treatment, whereas serum ALT, AST, and GGT activities were within reference ranges after 13 weeks and serum LDH activity was within the reference range after 19 weeks. The induction phase was concluded when Hct decreased to 30% and serum transferrin saturation was 31%. A total amount of 10.6 g of iron (53 mg/kg [24.1 mg/lb] of body weight) was removed from dolphin A during the induction phase.

Dolphin B did not have any clinical signs of illness and was not receiving any medications when phlebotomy treatment began. However, results of repeated (n = 38) CBCs and serum biochemical analyses performed throughout the 2-year period prior to phlebotomy treatment indicated the mean serum globulin (3.5 g/dL) and iron concentrations as well as the mean serum ALT, AST, GGT, and LDH activities were all markedly high (Table 1). In contrast with the values for dolphin A, the mean ESR (12 mm/h) and WBC count (9,842 cells/µL) were within reference ranges. Because of the larger body mass of dolphin B, the volume of blood removed during the first phlebotomy procedure was 2 L (approx 11% of the estimated blood volume). The volume of blood removed was increased to 3 L at week 21, and a similar volume was removed during each subsequent phlebotomy procedure until the end of induction phase. The duration of the induction phase for dolphin B was 24 weeks (25 phlebotomy procedures). After 10 weeks of phlebotomy treatment, serum iron concentration and AST activities in this dolphin were within reference ranges; after 19 weeks, serum ALT, GGT, and LDH activities were within reference ranges. The induction phase was concluded when the Hct decreased to 30% and serum transferrin saturation was 30.3%. A total amount of 18 g of iron (73 mg/kg [33.2 mg/lb] of body weight) was removed from dolphin B during the induction phase.

Dolphin C had no clinical signs associated with iron overload or leukocytosis. However, results of repeated (n = 29) CBCs and serum biochemical analyses performed throughout the 2-year period prior to phlebotomy treatment indicated the mean serum iron concentration (462 µg/dL), globulin concentra-

tion (3.6 g/dL), and ALT (129 U/L), AST (649 U/L), GGT (335 U/L), and LDH (522 U/L) activities were all markedly elevated. Mean WBC count (11,542 cells/μL) was elevated prior to phlebotomy treatment, but the ESR (11 mm/h) was within the reference range. The induction phase for dolphin C included 31 phlebotomy procedures during a period of 30 weeks. The volume of blood removed during the initial phlebotomy procedure was 2 L; this volume was increased to 3 L at week 28. Serum iron concentration was within the reference range after 5 weeks of treatment, but the serum transferrin saturation remained > 20% until week 24 of treatment. The serum activities of the liver enzymes gradually decreased to reference range values at various time points; abnormalities in serum LDH, AST, GGT, and ALT activities were not evident after 16, 28, 28, and 31 weeks, respectively. At the conclusion of the induction phase, Hct was 35.8%, serum iron concentration was 62 μg/dL, and serum transferrin saturation was 17.5%. A total amount of 27.1 g of iron (111 mg/kg [50.5 mg/lb] of body weight) was removed from dolphin C during the induction phase.

Results of simple linear regression[d] analysis revealed that the duration of the induction phase was a significant ($P < 0.001$) predictor of the rate of decrease in serum iron concentration; ALT, AST, GGT, and LDH activities; and percentage of serum transferrin saturation in all 3 dolphins. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in serum iron concentration were 0.81 for dolphin A, 0.39 for dolphin B, and 0.61 for dolphin C. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in serum ALT activity were 0.77 for dolphin A, 0.69 for dolphin B, and 0.90 for dolphin C. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in serum AST activity were 0.86 for dolphin A, 0.68 for dolphin B, and 0.85 for dolphin C. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in serum GGT activity were 0.73 for dolphin A, 0.65 for dolphin B, and 0.76 for dolphin C. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in serum LDH activity were 0.22 for dolphin A, 0.49 for dolphin B, and 0.75 for dolphin C. Adjusted R^2 values for the association between the duration of the induction phase and rate of decrease in percentage of serum transferrin saturation were 0.83 for dolphin A, 0.37 for dolphin B, and 0.65 for dolphin C.

No adverse clinical effects of treatment were detected in any of the dolphins during the phlebotomy treatments. Each dolphin performed training exercises normally. The peduncle PAVR site in each dolphin was used for phlebotomy on a weekly basis as many as 31 times; nevertheless, repeated blood removal procedures were not associated with adverse effects at the venipuncture site, and the vascular integrity of the peduncle PAVR was preserved.

After the completion of the induction phase of treatment and the start of the maintenance phase, blood samples were collected every 1 to 2 weeks for CBC, serum biochemical analysis, and determination of the percentage of serum transferrin saturation. After the start of the maintenance phase, Hct was within reference range (38% to 42%) at 10 weeks for dolphin A, at 5 weeks for dolphin B, and at 2 weeks for dolphin C.

The criteria for performing a phlebotomy procedure during the maintenance phase in any dolphin were high serum iron concentration and serum transferrin saturation > 60%. When these criteria were met, 2 L of blood was removed to prevent reaccumulation of body stores of iron. During the initial 6 months of the maintenance phase, 3 phlebotomy procedures were performed on dolphin A at 8- to 12-week intervals. For dolphins B and C, serum iron concentration and ALT and AST activities were within reference ranges and serum transferrin saturation was < 60% during the first 26 and 5 weeks, respectively, of the maintenance phase of treatment; therefore, these dolphins did not require additional phlebotomy procedures during those respective periods of the maintenance phase.

An ANCOVA[e] to compare values of selected hematologic and serum biochemical variables determined during the 2-year period preceding the induction phase and during the initial 6-month maintenance period following induction in dolphins A and B was conducted. In the preinduction and initial maintenance periods, 34 and 20 blood samples, respectively, were collected from dolphin A and 38 and 23 blood samples, respectively, were collected from dolphin B. Dolphin C was not included in the analysis because of the short period of postinduction evaluation (5 weeks), which resulted in limited data. A general linear model was used to control for the varying numbers of samples per period and intervals between the last feeding and collection of the blood samples. Significance was set at a value of $P < 0.05$.

For 6 months after the start of the maintenance phase, serum iron concentration and AST, ALT, LDH, and GGT activities in dolphins A and B were significantly (all values of $P < 0.001$) decreased, compared with preinduction values, and were within reference ranges (Table 1). Although the mean platelet counts during the 2-year preinduction period (dolphin A, 74.8×10^3 platelets/μL; dolphin B, 70.6×10^3 platelets/μL) and the 6-month maintenance period (dolphin A, 95.9×10^3 platelets/μL; dolphin B, 84.1×10^3 platelets/μL) were within the reference range (55 to 143×10^3 platelets/μL), there was a significant (dolphin A, $P < 0.001$; dolphin B, $P = 0.04$) increase in the platelet counts between the preinduction and initial maintenance periods.

The mean values for ESR and serum globulin concentration, potential indicators of inflammation, were also significantly (all values of $P < 0.001$) decreased and within reference ranges during the initial 6-month maintenance period for dolphins A (ESR, 4 mm/h; serum globulin concentration, 2.2 g/dL) and B (ESR, 1 mm/h; serum globulin concentration, 2.7 g/dL). Although the mean neutrophil counts during the 2-year preinduction period (dolphin A, 7,306 cells/μL; dolphin B, 7,120 cells/μL) and the 6-month maintenance period (dolphin A, 6,171 cells/μL; dolphin B, 5,443 cells/μL) were within the reference range (2,737 to 7,570 cells/μL), there was a significant (dolphin A, $P = 0.03$; dolphin B, $P < 0.001$) decrease in the neutrophil counts between the preinduction and initial maintenance periods. In both dol-

phins, mean WBC counts (dolphin A, 11,478 cells/μL; dolphin B, 7,601 cells/μL) were decreased during the initial maintenance period, compared with findings during the preinduction period; however, the decrease was only significant ($P < 0.001$) for dolphin B. The lack of a significant decrease in the mean WBC count for dolphin A may have been attributable to chronic lymphocytosis (mean lymphocyte count during the initial maintenance period, 3,764 cells/μL). Although not all the inflammatory indicators had mean values greater than the upper limits of the reference ranges prior to treatment, phlebotomy treatment resulted in clinically important and sustainable decreases in these inflammatory indicators.

Discussion

In the 3 bottlenose dolphins of this report, phlebotomy procedures were performed weekly and the removal of large amounts of circulating iron significantly decreased the dolphins' abnormally high serum iron concentrations; additionally, these treatments resulted in decreases in serum ALT, AST, GGT, and LDH activities and percentage of transferrin saturation, such that values were within reference ranges. The effects of phlebotomy treatment supported the diagnosis of iron overload in these 3 dolphins. The liver is the primary site for ALT activity in bottlenose dolphins; AST and LDH are widely distributed in tissues, including liver, heart, muscles, and kidneys, and GGT is concentrated in the kidneys.[8,9] Increases in serum ALT, AST, GGT, and LDH activities are generally believed to be indicative of primary hepatic disease in dolphins, but can also be indicative of extrahepatic diseases.[9] In each of the dolphins in this report, results of histologic examination of biopsy specimens confirmed excessive iron deposition in the liver; additionally, hepatitis was diagnosed in 2 of the dolphins. The markedly high serum AST, GGT, and LDH activities were also indicative of excessive iron deposition and cellular damage in other tissues as well.

Although the temporal and causative relationships of iron deposits and the development of liver disease in bottlenose dolphins is unknown, iron accumulation and overload in other species can be a primary hereditary disease or may develop secondary to diet, infection, toxicosis, and other disease processes.[10] Reported causes of liver disease in cetaceans include trematode infestation,[11,12] hepatitis B–like virus infection,[13] lead toxicosis,[14] toxoplasmosis,[15] and suspected acquired immunodeficiency.[16] Two separate population-based studies involving stranded cetaceans in the Canary Islands[17] and coasts of Italy[18] revealed that 47 of 135 and 10 of 25 animals, respectively, had nonspecific chronic active hepatitis and nonpurulent hepatitis. Although systemic hemosiderosis was detected in the cetaceans with lead toxicosis[14] and disseminated toxoplasmosis,[15] there are no reports of iron overload in cetaceans to our knowledge.

Iron overload has been detected in California sea lions[19] and Northern fur seals[20] and is recognized as a disease of concern in several species of zoo mammals,[10] including lemurs and other nonhuman primates,[21,22] rhinoceroses,[23] and tapirs.[24] In captive wildlife, iron overload is often attributed to consumption of nonnative diets, certain types of anemia, and other chronic inflammatory processes. In humans, hereditary hemochromatosis is a common autosomal recessive genetic disorder of iron metabolism in which excessive absorption of iron by gastrointestinal enterocytes leads to excess iron storage in multiple parenchymal tissues.[25] Mammals have no physiologic mechanisms to excrete body stores of iron, and iron depletion occurs only via blood loss, desquamation of cells, menstruation, and pregnancy.

During phlebotomy treatment, iron is removed from the body via repeated venipuncture. This causes mobilization of tissue stores of iron for incorporation into hemoglobin during erythropoiesis. Iron-chelating agents can be administered, and the bound iron is then excreted in urine; however, they lack the efficacy of phlebotomy treatment and are only used in patients that cannot tolerate blood loss.[5] Early diagnosis and treatment of hemochromatosis in humans greatly improves associated clinical symptoms, increases life expectancy, and resolves liver disease that has not progressed to irreversible cirrhosis.[26] In veterinary medicine, phlebotomy is a novel treatment that has been used with some success to treat lemurs[27] and horses[28] that have iron overload.

During phlebotomy treatment of humans with hemochromatosis, a volume of blood is removed weekly and Hct, hemoglobin concentration, MCV, and serum ferritin concentration and percentage of transferrin saturation are commonly monitored so that the treatment protocol can be altered as needed and the outcome of phlebotomy treatment can be evaluated. Iron depletion is evidenced by microcytic anemia and reduced serum ferritin concentration and percentage of transferrin saturation.[5,29] In humans, iron depletion is complete when serum ferritin concentration is 10 to 20 μg/L, hemoglobin concentration is < 11 g/dL, or Hct is < 33% for > 3 weeks.[5]

In the 3 dolphins of this report, up to 12 mL of blood/kg (5.5 mL/lb) of body weight was safely removed each week during the induction phase of phlebotomy treatment; adverse effects of phlebotomy were not detected. Safety of the phlebotomy treatments was evaluated by closely monitoring RBC indices, including Hct and hemoglobin concentration. Criteria for ending the induction phase (Hct ≤ 30% or serum transferrin saturation ≤ 20%, serum iron concentration within the reference range, and serum ALT and AST activities within reference ranges) were appropriate for this species—the phlebotomy protocol was safe and successfully used to remove excess body stores of iron from the dolphins.

Dolphin A developed a hypochromic macrocytic anemia that was attributed to the large number of nucleated RBCs in the blood. The large number of nucleated RBCs as well as the chronic progressive lymphocytosis may have been associated with a bone marrow disorder. Dolphins B and C developed hypochromic microcytic anemia that was consistent with iron deficiency. The hematologic responses (data not shown) of dolphins B and C to the phlebotomy treatment paralleled those in humans, in whom there is a transitory increase in MCV before the value decreases to 5% to 10% less than the pretreatment value.[29] In dolphins, as in humans, MCV

may be a useful and easily obtained measure for evaluating the development of iron-limited erythropoiesis and MCV values may be used as a criterion for beginning the maintenance phase of treatment.

Assessment of serum ferritin concentration is considered the most reliable method for monitoring the overall body stores of iron and the outcome of phlebotomy treatment, but the test requires species-specific antibodies and is not currently validated for cetaceans. However, the percentage of serum transferrin saturation can be useful for the diagnosis of iron storage disease and for monitoring phlebotomy treatment.[5,25] Typically, serum transferrin saturation is 33% in mammals, but wide diet-associated and diurnal fluctuations can occur.[10,29] Iron depletion in humans is achieved when the serum transferrin saturation is < 16%. When the criteria for conclusion of the induction phase were achieved in the dolphins of this report, the percentages of serum transferrin saturation had decreased from pretreatment values of 80% to 90% for all the dolphins to 33% for dolphin A, 30% for dolphin B, and 17.5% for dolphin C. For the population of healthy bottlenose dolphins ≥ 5 years of age that is managed by the United States Navy Marine Mammal Program, the mean ± SD for percentage of serum transferrin saturation is 50 ± 12% (n = 20). On the basis of these findings, evaluation of the percentage of serum transferrin saturation appears to be useful for diagnosis of iron overload and for monitoring phlebotomy treatment in dolphins.

The median interval between maintenance phlebotomy procedures in humans with hemochromatosis is 7.5 weeks (range, 5 to 18 weeks).[29] At the time of the last assessment, dolphin A had been in the maintenance phase for 6 months and had received 3 phlebotomy procedures at intervals of 8 to 12 weeks; dolphin B had been in the maintenance phase for 6 months and had not required any phlebotomy procedures; and dolphin C had been in the maintenance phase for only 5 weeks and had not required any phlebotomy procedures. Although the interval between phlebotomy treatments in dolphin A during the maintenance phase was similar to that in humans, the criteria for performing a maintenance phlebotomy procedure (serum iron concentration greater than the upper limit of the reference range and serum transferrin saturation > 60%) may be too conservative; by the time that serum iron concentration is abnormally high, detrimental iron overload in parenchymal organs may have already begun. Reliance on increases in percentages of serum transferrin saturation (ie, values > 60%) as a sole criterion to determine when to perform a maintenance phlebotomy procedure may prevent the onset of cellular damage and recurring increases in serum iron concentration and ALT and AST activities. For the dolphins of this report, the continuing treatment plan for the maintenance phase included monthly blood sample collections for assessments of serum iron concentration, ALT and AST activities, and percentage of serum transferrin saturation, the findings of which would be used to determine the need for phlebotomy treatment.

In the dolphins of this report, ESR and serum globulin concentration during the initial maintenance phase were lower than the values during the preinduction period. Additionally, platelet counts were higher during the initial maintenance phase, compared with the preinduction platelet counts. The fact that values of indicators of inflammation were within reference ranges following the induction phase of phlebotomy treatment suggests that iron overload had direct effects on serum globulin concentration and ESR. Additionally, the fact that serum iron concentration and ALT, AST, GGT, and LDH activities were within reference ranges following the induction phase of phlebotomy treatment supports an important role for iron overload in the chronic phase of hepatopathy in the 3 dolphins. Without a confirmed diagnosis of the underlying disease, determination of which developed first—hepatopathy or iron overload—is difficult. Follow-up histologic examinations of liver biopsy specimens would be necessary to determine whether the hepatitis in dolphins A and B resolved. However, the clinicopathologic data suggest that total body stores of iron were reduced to minimum physiologic amounts.

The cause of iron overload in dolphins is likely multifactorial and may include genetic, nutritional, and also infectious disease processes.[2] Although hereditary hemochromatosis in humans causes a primary iron overload disorder that may lead to liver damage, secondary iron overload can be a result of primary disorders of the liver and other factors such as viral hepatitis, ineffective erythropoiesis, and alcohol consumption.[30] Regardless of the cause, excess stores of iron can result in clinical abnormalities that can be resolved with the removal of iron via phlebotomy treatment. For example, in humans with chronic active hepatitis C virus infection, serum ALT activity is decreased, compared with values prior to phlebotomy treatments, and iron deposits are removed from the liver via phlebotomy treatments; however, the histologic abnormalities in the liver are not resolved.[31]

As a preventative measure, vitamin and mineral dietary supplements that contain iron or ascorbic acid (vitamin C) should be used with caution in healthy dolphins. Ascorbic acid enhances the absorption of dietary iron,[32] and high-quality fish contain adequate amounts of iron, thereby eliminating the need for administration of supplemental iron. Also, a recent study[33] revealed that gastric pH may play a role in iron absorption and maintenance of plasma iron concentrations in bottlenose dolphins. Thus, treatment with antacids to increase gastric pH may reduce iron absorption in dolphins with iron overload.

Although the causes of iron overload and hepatopathy in the 3 bottlenose dolphins of this report remained unknown, the use of a novel treatment that has been rarely used in animals other than humans successfully decreased serum iron concentration and ALT and AST activities to reference range values. Evaluation of additional dolphins with iron overload that undergo phlebotomy treatment for longer postinduction periods are needed to refine the phlebotomy protocol and assess the duration and success of maintenance phlebotomy treatment.

a. Tru-cut, Allegiance Healthcare Corp, McGaw Park, Ill.
b. Hospira, Lake Forest, Ill.
c. Vacuum Collection Unit, Baxter, Deerfield, Ill.

d. PROC REG, SAS, version 8e, SAS Institute Inc, Cary, NC.
e. PROC GLM, SAS, version 8e, SAS Institute Inc, Cary, NC.

References

1. Venn-Watson S, Jensen ED, Ridgway SH. Effects of age and sex on clinicopathologic reference ranges in a healthy managed Atlantic bottlenose dolphin population. *J Am Vet Med Assoc* 2007;231:596–601.
2. Venn-Watson S, Smith CR, Jensen ED. Assessment of increased serum aminotransferases in a managed Atlantic bottlenose dolphin (*Tursiops truncatus*) population. *J Wildl Dis* 2008;44:318–330.
3. Venn-Watson SK, Ridgway SH. Big brains and blood glucose: common ground for diabetes mellitus in humans and healthy dolphins. *Comp Med* 2007;57:390–395.
4. Dold C, Ridgway S. Cetaceans. In: West G, Heard DJ, Caulkett N, eds. *Zoo animal and wildlife immobilization and anesthesia*. Ames, Iowa: Blackwell Publishers, 2007;485–496.
5. Barton JC, McDonnell SM, Adams PC, et al. Management of hemochromatosis. Hemochromatosis Management Working Group. *Ann Intern Med* 1998;129:932–939.
6. Ridgway SH, Johnston DG. Blood oxygen and ecology of porpoises of three genera. *Science* 1966;151:456–458.
7. Crosby WH, Conrad ME Jr, Wheby MS. The rate of iron accumulation in iron storage disease. *Blood* 1963;22:429–440.
8. Geraci JR, St Aubin DJ. Tissue sources and diagnostic value of circulating enzymes in cetaceans. *J Fish Res Board Can* 1979;36:158–163.
9. Reidarson TH, McBain J, Dalton LM. Lactate dehydrogenase isoenzymes patterns in cetaceans. *J Zoo Wildl Med* 1999;30:228–234.
10. Lowenstine LJ, Munson L. Iron overload in the animal kingdom. In: Fowler ME, Miller RE, eds. *Zoo and wild animal medicine: current therapy 4*. 4th ed. Philadelphia: WB Saunders Co, 1999;260–268.
11. Resendes AR, Juan-Sallés C, Almeria S, et al. Hepatic sarcocystis in a striped dolphin (*Stenella coeruleoalba*) from the Spanish Mediterranean coast. *J Parasitol* 2002;88:206–209.
12. Dailey M, Stroud R. Parasites and associated pathology observed in cetaceans stranded along the Oregon coast. *J Wildl Dis* 1978;14:503–511.
13. Bossart GD, Bawner TA, Cabal C, et al. Hepatitis B-like infection in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). *J Am Vet Med Assoc* 1990;196:127–130.
14. Shlosberg A, Bellaiche M, Regev S, et al. Lead toxicosis in a captive bottlenose dolphin (*Tursiops truncatus*) consequent to ingestion of air gun pellets. *J Wildl Dis* 1997;33:135–139.
15. Resendes AR, Almeria S, Dubey JP, et al. Disseminated toxoplasmosis in a Mediterranean pregnant Risso's dolphin (*Grampus griseus*) with transplacental fetal infection. *J Parasitol* 2002;88:1029–1032.
16. Bossart GD. Suspected acquired immunodeficiency in an Atlantic bottlenosed dolphin with chronic-active hepatitis and lobomycosis. *J Am Vet Med Assoc* 1984;185:1413–1414.
17. Jaber JR, Perez J, Arbelo M, et al. Immunophenotypic characterization of hepatic inflammatory cell infiltrates in common dolphins (*Delphinus delphis*). *J Comp Pathol* 2003;129:226–230.
18. Di Guardo G, Agrimi U, Morelli L, et al. Post mortem investigations on cetaceans found stranded on the coasts of Italy between 1990 and 1993. *Vet Rec* 1995;136:439–442.
19. Garcia AR, Montali RJ, Dunn JL, et al. Hemochromatosis in captive otariids, in *Proceedings*. Am Assoc Zoo Vet Int Assoc Aquat Anim Med Joint Conf 2000;197.
20. Mazzaro LM, Dunn JL, St Aubin DJ, et al. Serum indices of body stores of iron in Northern fur seals (*Calloshrinus ursinus*) and their relationship to hemochromatosis. *Zoo Biol* 2004;23:205–218.
21. Gonzales J, Benirschke K, Saltman P, et al. Hemosiderosis in lemurs. *Zoo Biol* 1984;3:255–265.
22. Miller GF, Barnard DE, Woodward RA, et al. Hepatic hemosiderosis in common marmosets, *Callithrix jacchus*: effect of diet on incidence and severity. *Lab Anim Sci* 1997;47:138–142.
23. Kock N, Foggin C, Kock MD, et al. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): a comparison of free ranging and recently captured with translocated and captive animals. *J Zoo Wildl Med* 1992;23:230–234.
24. Bonar CJ, Trupkiewicz JG, Toddes B, et al. Iron storage disease in tapirs. *J Zoo Wildl Med* 2006;37:49–52.
25. Smith LH. Overview of hemochromatosis. *West J Med* 1990;153:296–308.
26. Tavill AS. Diagnosis and management of hemochromatosis. *Hepatology* 2001;33:1321–1328.
27. Crawford GC, Andrews GA, Chavey PS, et al. Survey and clinical application of serum iron, total iron binding capacity, transferrin saturation, and serum ferritin in captive black and white ruffed lemurs (*Varecia variegate variegate*). *J Zoo Wildl Med* 2005;36:653–660.
28. Pearson EG, Hedstrom OR, Poppenga RH. Hepatic cirrhosis and hemochromatosis in three horses. *J Am Vet Med Assoc* 1994;204:1053–1056.
29. Bolan CD, Conry-Cantilena C, Mason G, et al. MCV as a guide to phlebotomy therapy for hemochromatosis. *Transfus Med* 2001;41:819–827.
30. Kohgo Y, Ikuta K, Ohtake T, et al. Iron overload and cofactors with special reference to alcohol, hepatitis C virus infection and steatosis/insulin resistance. *World J Gastroenterol* 2007;13:4699–4706.
31. Hayashi H, Takikawa T, Nishimura N, et al. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. *Am J Gastroenterol* 1994;89:986–988.
32. Hallberg L, Brune M, Rossander L. Effect of ascorbic acid on iron absorption from different types of meals. Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum Nutr Appl Nutr* 1986;40:97–113.
33. Mitchell MA, Solangi MA, Clemons-Chevis CL, et al. Relationship between plasma iron concentration and gastric pH in captive adult bottlenose dolphins (*Tursiops truncatus*). *Am J Vet Res* 2008;69:900–903.